# Valorization of Date Syrup by the Production of Lipopeptide Biosurfactants by a *Bacillus mojavensis* BI2 strain: Bioprocess Optimization by Response Surface Methodology and Study of Surface Activities

**Mnif Inès** ( inesmnif2011@gmail.com )
National School of Engineering of Sfax

**Amir Bouallegue**
Laboratoire d'Amélioration des Plantes et de Valorisation des Agro-ressources, Ecole Nationale d'Ingénieurs de Sfax, Tunisie. Bioréacteur couplé à un ultra filtra, Ecole Nationale D'Ingénieurs de Sfax, Université de Sfax, Tunisie

**Salwa Mekki**
Laboratoire d'Amélioration des Plantes et de Valorisation des Agro-ressources, Ecole Nationale d'Ingénieurs de Sfax, Tunisie. Faculté des Sciences de Gabes, Université de Gabes, Gabes, Tunisie

**Dhouha Ghribi**
Institut Supérieur de Biotechnologie de Sfax, Université de Sfax, Tunisie

**Research**

**Keywords:** Lipopeptide, Date Syrup Valorization, Surface Activity, Optimisation, Characterization, Stability

**Posted Date:** February 15th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-220287/v1

# Abstract

Lipopeptides Biosurfactants are natural surface-active compounds produced by a variety of microorganisms. They have great interest in environmental, biomedical and agro-industrial fields. However, the high cost of culture media and the low yield of production limit their large-scale production and application. The development of efficient and cost-effective bioprocess became of a great interest for the improvement of the yield of biosurfactants and the decrease of production cost. In this aim, we applied the response surface method to optimize an economic biosurfactant production by a newly isolated strain B. mojavensis BI2 on date syrup called “Luegmi” as unique carbon and nitrogen source. Using a Box-Bhenken design, we studied the effect of three independent variables on lipopeptide production; Leugmi concentration, Na 2 HPO 4 and incubation time. The results of this study showed that Leugmi concentration at 25%, Na 2 HPO 4 at 0.1% and incubation time of 24 hours were optimal conditions for biosurfactant production, with a maximum surface tension decreasing capacity of 55% corresponding to 27 mN/m and oil dispersing of 30 cm 2 corresponding to a diameter of 6 cm. Preliminary characterization of the biosurfactant produced on Luegmi by UV-Spectra and Thin Layer Chromatography showed its lipopeptide characters. Physic-chemical characterization of the produced lipopepetide on Leugmi showed its great surface activities and stabilities at different pH, temperature and salts concentration. The results of this study suggested that Leugmi, an agricultural byproducts can be used as a low-cost substrate to enhance the yield of lipopeptide biosurfactants with great surface activities for potential environmental application.


# Background

Biosurfactants are mainly produced by micro-organisms that are, in general, yeasts, fungi or bacteria, developing aerobically in an aqueous medium containing one or more sources of carbon, such as carbohydrates, oils or hydrocarbons. Generally, the main physiological role of biosurfactants is to allow microorganisms to develop on insoluble substrates by reducing the TIF between water and substrate, making it more easily accessible (Mnif &Ghribi 2015a-b). Most microorganisms with the capacity to produce biosurfactants are usually isolated from soils contaminated with hydrophobic molecules (hydrocarbons or vegetable oils). Biosurfactants, being highly biodegradable, eco-friendly along with a high stability towards various physic-chemical conditions, they have potential wide applications in various fields including environment and bioremediation (Mnif &Ghribi 2015a-a, Mnif &Ghribi 2015b, Mnif &Ghribi 2015c) (Olasanmi &Thring 2018).

Lipopeptides are among the most popular biosurfactants. Surfactin, iturin and fengycin are members of lipopeptide family, which is considered as the most powerful and known biosurfactants. They are well known by their ability to emulsify, solubilize and mobilize hydrocarbons enhancing therefore their biodegradation (Mnif et al. 2014, Mnif et al. 2015a, Mnif et al. 2013a). Moreover, they have the capacity to improve dyes biodecolorisation (Mnif et al. 2015b, 2015c, Mnif et al. 2016a). Indeed, environmental application needs great quantities of biosurfactant preparation and the success of the use of bio-surfactants depends on the costs of their production. Generally, the cost of nutritional components has a significant impact on the final cost of production. It is estimated that the raw material represents 10-30% of the final cost of production in most biotechnological processes (Singh et al. 2019). The economic production of biosurfactants and the improvement of production yields is a major concern of biotechnologists. They can be achieved by 3 different

strategies: the use of low-cost substrates: economic substrate; the development of more efficient processes, such as optimization of production conditions: optimization of nutritional and physic-chemical parameters and the development and use of recombinant and over-productive strains (Singh et al. 2019); (Dang et al. 2019); (Shi et al. 2018); (Hu et al. 2019).

Generally, several nutritional parameters can influence the production of biosurfactants such as carbon source, nitrogen source and mineral elements (Beltran-Gracia et al. 2017); (Singh et al. 2019); (Nurfarahin et al. 2018). Generally, lipopeptides can be produced using a wide range of carbohydrate-like hydrophilic carbon substrates and hydrophobic substrates separately or on the two substrates at the same time (Mnif &Ghribi 2015b). Biosurfactants can be produced using a wide range of carbohydrate-like hydrophilic carbon substrates (Zhang et al. 2016); (Ghazala et al. 2017); (Hmidet et al. 2017); (Phulpoto et al. 2020) or hydrophobic substrates such as vegetable oils and hydrocarbons (Ndlovu et al. 2017); (Ohadi et al. 2017); (Patowary et al. 2017) and this either as the sole source of carbon or in combination (Sarubbo et al. 2016); (Joy et al. 2017). However, glucose remains the primary source for the production of biosurfactants (Eswari et al. 2016). Nevertheless, other sugars like sucrose can support an interesting lipopeptide biosurfacatnt production (Liu et al. 2012); (Singh et al. 2014). In addition to carbon source, different organic or inorganic nitrogen sources can be used to produce biosurfactants. Yeast extract, tryptone and glutamic acid are well used as organic nitrogen sources supporting higher production yield of lipopeptides (Eswari et al. 2016); (Beltran-Gracia et al. 2017). Complex compounds such as soybean flour peptone and casein acid hydrolysate have been assayed too (Beltran-Gracia et al. 2017). Moreover, several inorganic nitrogen compounds have been tested in lipopeptide production like ammonium nitrate, ammonium sulphate, sodium nitrate, urea and glutamic sodium (Beltran-Gracia et al. 2017); (Moshtagh et al. 2019);(Phulpoto et al. 2020). The nitrogen source may in some cases affect the nature of the biosurfactant produced (Mnif &Ghribi 2015b) . On the other hand, it should be noted that trace elements like iron are an important factor in the production of biosurfactants (Beltran-Gracia et al. 2017). (Wei &Chu 2002), (Abushady et al. 2005) and (Wei et al. 2004) show that the addition of Manganese, the addition of Iron and the simultaneous addition of Iron and Manganese respectively stimulate the production of surfactin by *B. subtilis*. Moreover, supplementation of the culture medium with divalent cations such as Iron and Magnesium stimulates the production of Iturin A by *B. amyloliquefaciens* B128 in a dose-dependent manner (Lin et al. 2007). As suggested by (Bartal et al. 2018), the addition of metal ions $Mn^{2+}$, $Cu^{2+}$ and $Ni^{2+}$ in the media have great influence on the surfactin variant composition produced by *Bacillus subtilis* strain.

Additionally, economical production is of great interest for the decrease the cost of production and the maximization of the production yield. Several food industry wastes were used in the production of surfactant lipopeptides such as millet flour (Ghribi et al. 2012), sesame peel flour (Mnif et al. 2013a), rice bran (Shih et al. 2008), tuna fish flour and potato waste flour (Mnif et al. 2013c), soybean flour (Wang et al. 2008); (Kim et al. 2009), soybean sauce (Cho et al. 2009), potato residues (Wang et al. 2008), brewery waste (Moshtagh et al. 2019), papaya waste (Soares et al. 2018), molasses and whey (Rodrigues et al. 2006); (Joshi et al. 2008); (Hippolyte et al. 2018) and butter milk and poultry-transforming wastes (Zouari et al. 2019a). These raw materials are very rich in sugars corresponding to carbon substrates favorable for the production of lipopeptides. In particular, some vegetable oils and waste generated from oil refinery are used in the production of lipopeptides such as peanut seed refining residue (Luna et al. 2015), palm oil refining residue (Chooklin et al. 2013) and sunflower oil (Mani et al. 2016) and olive-mill waste (Zouari et al. 2014), (Ramírez et al. 2015). Additionnally, its well demonstrated that the nature of the substrate can influence the diversity of the produced

lipopeptides. (Paraszkiewicz et al. 2018) studied lipopeptide production two *Bacillus subtilis* strains. They proved that the variability of the renewable natural resources used (brewery wastewaters, beet molasses and apple peels extract supplemented with yeast extract, peptone or carrot peels extract) have great influence on the production level and the structural diversity of synthesized lipopeptides (Paraszkiewicz et al. 2018). The bioconversion of agro-industrial waste and food by-products to value added secondary active metabolites like biosurfactants permit the valorisation and recycling of waste that pose problems of discharge in the nature. In addition, it permit to decrease the cost of production.

In addition to nutritional parameters, some physicochemical factors such as the incubation temperature, the initial pH of the culture medium and the rate of agitation can influence the production of lipopeptides (Beltran-Gracia et al. 2017); (Moshtagh et al. 2019); (Phulpoto et al. 2020). Generally, moderate agitation in the range of 150 to 200 rpm promotes good production of biosurfactants (Mnif &Ghribi 2015b). On the other hand, the production of surfactant lipopeptides can be achieved in a temperature range of 25 to 45°C (Mnif &Ghribi 2015b). The initial pH of the culture medium may also play a decisive role in the production of lipopeptides by some strains. Generally a neutral pH is favorable for optimal biosurfactant production in most strains (Mnif &Ghribi 2015b).

Besides the different nutritional and physic-chemical parameters affecting lipopeptide production, optimization of the different factors by the experimental methodology remains the best strategy to maximize biosurfactant production (Bertrand et al. 2018); (Singh et al. 2019); (Moshtagh et al. 2019); (Phulpoto et al. 2020). Therefore, in our study aiming to reduce the cost of biosurfactant production by a newly isolated strain *B. mojavensis* BI2, we choose the use of an agriculture byproduct along with the application of planning experimental methodology to optimize the yield of production. *B. mojavensis* BI2 was isolated in our laboratory from an oasis contaminated soil. The produced biosurfactant was able to decrease the Surface Tension to about 27.4 mN/m with a Critical Micelle Concentration of 200 mg/L; disperse motor oil to about 113 $cm^2$ at 1000 mg/L with a potential foaming activity of about 75% at 800 mg/L. In the first part of the present work, we tried to produce BI2 lipopeptide on “Luegmi” with the application of response surface methodology as an efficient tool to optimize the yield of production. In the second part of the present work, we tried to confirm the lipopeptide nature of the produced biosurfactant along with the study of the effect temperature, pH and salinity on the surface activities of the produced biosurfactant.

# Methods

### *Chemical product*

Motor oil used in this study was obtained from a local Mechanic’s station (Sfax, Tunisia). SDS (anionic surfactant) and Tween 80 (non-ionic surfactant) were purchased from Sigma and dissolved in distilled water. They were tested as synthetic chemical surfactants to perform the physicochemical tests in parallel with the biosurfactants preparation.

### *Microorganism*

The *Bacillus mojavensis* BI2 (MW130250) used in the present work was isolated in our laboratory from Tunisian Hydrocarbon contaminated soil. This strain was selected on the basis of the high hemolytic, surface

tension decrease capacity, oil dispersion and foaming activities of its biosurfactant.

### *Optimization of BI2 Lipopeptide Production Using Date Syrup "Luegmi" Under Submerged fermentation*

#### Inoculum and cultivation conditions

The inocula were prepared as described by (Mnif et al. 2013b). It was used to inoculate the production medium composed only of Luegmi, Yeast Extract and $Na_2HPO_4$ at the proportions given by the experimental design given in Table 1. The initial pH values of the media were adjusted to 7.0. Cultures medium were incubated for 24 hours under shaking at 150 rpm. Then, the cultures were centrifuged at 8000 rpm for 10 min and the resultant supernatant was used for the quantification of biosurfactant production, their extraction and analysis.

#### Design of the experiments

The experimental methodology was designed to formulate a new economic medium required for the optimum biosurfactant production of *B. mojavensis* BI2 under submerged fermentation. Experimental designs were modeled to optimize the production yield. Preliminary studies showed that the quantity Luegmi, yeast extract and $Na_2HPO_4$ significantly affected biosurfactant production. Therefore, to determine the optimum levels of these variables, to predict the possible interaction between the selected factors, and to enhance biosurfactant production, a Box-Bhenken design was adopted. It was generated using Nemrod-W Version 2007 software (LPRAI, Marseille, France). Each variable was assessed at three coded levels (−1, 0, + 1). A total of 16 experiments were conducted, including $2^3$ full factorial design experiments (runs N°1 to 8), 4 axial points (runs N°9 to 12) and 4 replicates in the domain centre (runs N°13 to 16) to estimate the variability of the experimental results (Table 1). The response values (Y) used in each trial was the average of the duplicates (Table 1). The production of biosurfactant is monitored by Surface Tension measurement and oil dispersant activity.

### *Statistical Analysis and Modeling*

The data obtained from the response surface methodology with regard to *B. mojavensis* BI2 biosurfactant production were subjected to analysis of variance (ANOVA). ANOVA permit to check the errors and the significance of each parameter. Surface tension reduction and the area of oil displacement were taken as responses ($Y_1$ and $Y_2$ respectively). The data were then subjected to a multiple regression analysis to obtain an empirical model that could relate the response measured to the independent variables. The behavior of the system was explained by the following quadratic equation:

$$Y1 = b_0 + b_1 * X_1 + b_2 * X_2 + b_3 X_3 + b_{1\text{-}1} * X_1^2 + b_{2\text{-}2} * X_2^2 + b_{3\text{-}3} * X_3^2 + b_{1\text{-}2} * X_1 * X_2 + b_{1\text{-}3} * X_1 * X_3 + b_{2\text{-}3} * X_2 * X_3$$

Where $X_1$, $X_2$ and $X_3$ were the coded factors studied (Table 2), $b_0$ the intercept, $b_1$, $b_2$ and $b_3$ the linear coefficients, $b_{1\text{-}1}$, $b_{2\text{-}2}$ and $b_{3\text{-}3}$ the squared coefficients and $b_{1\text{-}2}$, $b_{1\text{-}3}$ and $b_{2\text{-}3}$ the interaction coefficients.

The model coefficients were estimated using multi-linear regression by the statistical software package (Nemrod-W by LPRAI Marseilles, France) as described in our previous works (Ghribi et al. 2012, Mnif et al. 2013d, Mnif et al. 2013c, Mnif et al. 2013b). The multiple regression analysis permitted to evaluate the

statistical significance of the model based upon the F test with unequal variance ($p < 0.05$) The regression analyses were conducted on the experimental data and the response surface graphs were plotted. They represented the system behavior. The two dimensional graphical representation called the iso-response contour plot described the individual and cumulative effects of the variables. Also, they permit to predict the possible correlations that would exist between them.

### *Biosurfactant recovery*

For test motor oil dispersion study and surface tension measurement, lipopeptide biosurfactants were partially purified during three consecutive cycles of acid precipitation–dissolution (Mnif et al. 2013b, Mnif et al. 2013a). Supernatant, collected by centrifugation of the culture media at 10.000 rpm for 10 min, was subjected to two cycles of acid precipitation-dissolution. The cell free supernatant was acidified to pH 2.0 by adding 6 N HCl and incubated overnight at 4°C for the precipitation of biosurfactants products. The precipitated biosurfactants were then collected by centrifugation at 8000 rpm and 4°C during 20 min, washed twice with acid distilled water (pH = 2) to eliminate any impurities, dissolved in distilled water and the pH was adjusted to 8.0 by 1 N NaOH to dissolve the most lipopeptide compounds. This operation was repeated once time and the final dissolved extract was lyophilized. This serves as crude lipopeptide preparation to perform the entire biochemical study (TLC, UV and FTIR analysis) and physic-chemical characterization test by monitoring the surface tension measurement test, the oil dispersion capacity and the foaming activity.

## Biochemical characterization of the produced biosurfactant

### Organic extraction and TLC analysis

The organic extract was recovered from the crude biosurfactant preparation after three extractions with an equal volume of an ethyl acetate/methanol mixture (8:1, v/v). Organic phases were combined, passed over anhydrous sodium sulfate and concentrated in a rotary vacuum evaporator (Büchi, Flawil, Switzerland). For TLC analysis, crude extract sample was dissolved in methanol, spotted on silica plates (ALUGRAM® SIL G/UV254, Macherey-Nagel, Düren, Germany). Chloroform/methanol (65:35) was used as the mobile phase. The resulting spots on the TLC were viewed by spraying with ninhydrine (Sigma, St. Louis, MO) for amine groups; Molish solution for sugar observation; and iodine vapors for lipid observation (Coronel-León et al. 2015).

### Ultra-Violet spectra realization

UV–visible spectroscopy was performed to define different functional group of the produced biosurfactant. Study was conducted on crude preparation obtained after three cycles of acid precipitation-neutralization. After that, a few quantity of the crude biosurfactant was dissolved in water and their absorption properties were checked in UV and visible range in a Perkin-Elmer double beam UV–visible spectrophotometer as described by (Mukherjee et al. 2009). Samples were taken in quartz cuvette and scan was performed from 600 to 190 nm range by acquiring data at intervals of 10 nm. After that, optical density versus the wavelength was plotted.

## FTIR analysis

In order to determine the biochemical nature of the biosurfactant produced by BI2, we proposed to create an infrared spectrum using a Shimadzu FTIR spectrophotometer (model 8400S). The technique determines the

functional groups and chemical bonds present in the biosurfactant molecule (Biniarz et al. 2017). The sample is prepared by homogeneous dispersion of 1 mg of biosurfactant extract in potassium bromide granules (Merck, USA). The IR absorption spectrum was obtained using an integrated tracer. It was collected over a range of 450 to 4500 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. The spectral data was the average of 50 scans over the entire range covered by the instrument.

### *Functional characterization of crude lipopeptide preparation*

#### Surface tension measurement

The surface tension decrease of the crude lipopeptide preparation and the cell free supernatant was measured, according to the Du Noüy Ring method by a model Tensiometer Sigma 700 at room temperature (Mnif &Ghribi 2015a-a, Pacwa-Płociniczak et al. 2011). Distilled water was used as negative control.

#### Study of oil dispersion activity

Dispersion capacity of motor oil was studied by oil displacement test. Results were expressed as the surface of the clear zone. The oil displacement test was done by adding 40 mL of distilled water to a Petri dish with a diameter of 15 cm. After that 20 µL of motor oil was dropped onto the surface of the water in the center of the petri dish, followed by the addition of 10 µL of culture broth supernatant. The area of the clear halo on the oil surface was measured and compared with a negative control using 10 µl distilled water (Rodrigues et al. 2006).

### *Effects of physic-chemical factors on surface tension reduction and oil dispersion activities and stabilities*

To study the effect of different physicochemical factors on ZNI5 biosurfactant, oil dispersion and surface tension reduction activities were measured at different pH, Temperature and NaCl concentration values (Mnif et al. 2013b, Mnif et al. 2013a). Experiments were performed as described above for test displacement assay with 0.1 % biosurfactant solution. For the surface activity determination, experiments were conducted at the Critical Micelle Concentration. The effect of pH buffer on the two activities was evaluated at different pH values ranging from 2.0 to 10.0 using glycine–HCl buffer (pH 2.0–3.0), acetate buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–8.0) and glycine–NaOH buffer (pH 9.0–10.0) (all at a final concentration of 20 mM) (Mnif et al. 2013b). Results were expressed as residual activity towards dispersion activity at neutral pH. For the determination of salt effect, different quantities of NaCl were dissolved in buffer solutions to adjust the salt concentration of test samples to 0, 0.25, 0.5, 1, 2.5, 5, 7.5 and 10 % (weight/volume). Results were expressed as residual activity towards activities without salt addition. Also, we evaluated the activities at different temperature ranging from 4°C to 80°C. Residual activity was determined towards the activity measured at ambient temperature.

Similarly, the stability of the biosurfactant was evaluated after pre-incubation at different pH buffers, different salt concentration and different temperature by measuring the oil dispersion and the surface tension reduction activities. All results were expressed by relative activity towards biosurfactants activities without incubation.

## Results And Discussion

## *Formulation of an economic media supporting Bacillus mojavensis BI2 biosurfactant production: Use of Response Surface Methodology to optimize the production yield*

Generally, the success of the use of biosurfactants depends on the costs of their production. It is estimated that the raw material represents 10-30% of the final cost of production in most biotechnological processes, which has a significant impact on the final cost of the product. During this work, we were able to effectively produce biosurfactants using a food by-product “Leugmi” extracted from date palm. This juice extracted from the heart of the date palm is harvested directly from a gargoulette placed on the top of the palm. The technique is meticulous: prune the tree with a sharp tool, dig in the center to the tender part of the heart and collect the sap called «Leugmi». It is a very pleasant hygienic drink rich in sugars (about 12%) and minerals with a low content of nitrogen and fat. Sugars are mainly composed of sucrose, a simple sugar widely used for the production of lipopeptide biosurfactants by many strains (Kanna et al. 2014, Liang et al. 2017, Liu et al. 2012, Pereira et al. 2013, Singh et al. 2014). Thus, Luegmi can support the production of biosurfactants as substitutes for commercial sugars. Preliminary studies show the decrease of surface tension and the appearance of oil displacement activity when cultivating *B. mojavensis* BI2 on the date juice. However, adding nitrogen sources and minerals does not improve the production of biosurfactants. Therefore, results have shown the potential use of the developed date juice called “Luegmi” as a unique source of Carbon and Nitrogen to produce biosurfactants. Nevertheless, the addition of $Na_2HPO_4$ owing a buffer effect improves significantly biosurfactant production. In fact, $Na_2HPO_4$ neutralizes the acidic pH of Luegmi, which can inhibit the production of metabolites. Thus, $Na_2HPO_4$-supplemented Luegmi can be used as a ideal medium for the production of biosurfactant in a submerged fermentation system by strain BI2. In this aim, a Box-Bhenken design has been adopted in order to formulate a new economic medium based on Luegmi to obtain an optimum biosurfactant production. Production was followed by measurement of the decrease in Surface Tension as well as the dispersant activity of motor oil in the fermentation supernatant. We note that the values presented are the average of the results of 2 independent trials with 2 replicates for each trial. Table 1 shows the experimental and predicted responses. The percentage of Surface Tension decrease ranged from 26.2 to 54.86 % while the value oil dispersion areas are ranging from 0.19 to 32.15 $cm^2$. This wide variability in observed responses suggests the right choice of factors and their assigned levels. For the analysis of the results, a cubic response surface model was used. So, the following regression equations (Y1 and Y2) that reflect the empirical relationships between production yield estimated by the measure of surface tension reduction and the area of oil displacement and the test variables in coded units were obtained.

$$Y1 = 47.495 + 1.276*X_1 + 9.418*X_2 - 0.564\,X_3 + 2.180*X_1^2 - 7.398*X_2^2 - 1.740*X_3^2 + 5.732*X_1*X_2 - 1.735*X_1*X_3 - 1.468*X_2*X_3$$

$$Y2 = 14.420 + 5*X_1 + 10.471*X_2 - 1.006*X_3 - 3.511*X_1^2 - 0.619*X_2^2 - 1.686*X_3^2 + 6.665*X_1*X_2 - 4.120*X_1*X_3 - 0.147*X_2*X_3$$

With Y1 denotes the percentage of Surface Tension decrease; Y2 denotes the dispersion surface and $X_1$, $X_2$ and $X_3$ are respectively the coded values of Luegmi concentration, $Na_2HPO_4$ quantity and the time of

fermentation. Combinations of factors (such as $X_1X_2$) represent an interaction between individual factors and $X_1^2$; $X_2^2$; $X_3^2$ represent the double interactions of factors 1; 2 and 3.

The significance of each coefficient was determined by Student's t-test. Student's t distribution, the corresponding p values and the parameter estimates are listed in Table 2. The p values were used to prove the significance of each of the coefficients. Indeed, as shown in Table 4, X1 (Luegmi concentration) affect significantly the surface tension decrease only, X2 ($Na_2HPO_4$ quantity) had significant effects on the surface tension decrease and the area of oil displacement. However, the variation of the time of fermentation had no significant effect on the two studied response and therefore biosurfactant production.

The fit of the model was evaluated by the determination of the coefficient $R^2$, which had a value of 0.921 for the percentage of Surface Tension decrease and 0.944 for surface tension dispersion area. This suggests an adequate adjustment of the quadratic model to the experimental data and indicates that the model could explain 92.1 % and 94.4 % of the variability in the response for the first and the second response respectively. The closer the value of $R^2$ to 1, the better the model would explain the variability between the experimental and model predicted values (Mnif et al. 2013d, Mnif et al. 2013b, Mnif et al. 2013a). The statistical significance of the regression equation was checked by Fisher's F test. The results of ANOVA analysis presented in Table 3 showed that the regression model was significant and the lack of fit was insignificant. This analysis shows that the total sum of the squares of the deviations from the average estimated with 15 degrees of freedom is divided into two sums of squares. The first, estimated with 9 degrees of freedom, is due to regression; the second, estimated with 6 degrees of freedom, is due to residual variation. On the other hand, the analysis of variances shows that the regression was moderately significant for the two responses studied (99%) and the lack of validity is insignificant. Moreover, these results show that the model adopted is adequate to predict the choice of variables to be used.

The three-dimensional response surface curve and their respective contour plot obtained according to this analysis are shown in Fig. 1, 2 and 3. This curve represents the interaction between the selected factors, Luegmi concentration, $Na_2HPO_4$ quantity and fermentation time one-to-one. They are used to study the effects of the variation of the factors in the domain studied and, consequently, to determine the optimal experimental conditions. In fact, the elliptical nature of the contour plots indicated the significance of the interactions between the corresponding variables (Mnif et al. 2013d, 2013c, 2013b, 2013a). As mentioned previously, the enhancement of incubation time had no significant effect on biosurfactant production. Thus, we kept a minimum incubation time of 24 hours as a constant and searched the optimal values of the two other factors permitting an optimal biosurfactant production by BI2. As observed in Figure 1 presenting the variation in the % of ST decrease as a function of the concentration of Luegmi and $Na_2HPO_4$ quantity, the increase in the amount of $Na_2HPO_4$ significantly improves the abatement of ST. Also, low concentrations of Luegmi cannot support an optimal production of biosurfactants. Indeed, a reduction in the ST of the order of 54.99 % (27 mN/m) with a dispersion area of the order of 30.61 $cm^2$ can be achieved by working with 25.22 % Luegmi and 0.1 % $Na_2HPO_4$. The analysis of the iso-response curve presented in Figure 2 describing the evolution of the ST decreasing power as function of the Luegmi concentration and the time of fermentation and a constant $Na_2HPO_4$ fixed at 0.1 % value permit the achievement of the same optimal conditions. It demonstrated that

working with 25 % Luegmi during 24 hours of fermentation permit to support a maximum biosurfactant production.

Regarding the oil dispersion capacity and to confirm our optimal conditions, we studied the variation in the area of motor oil dispersion as a function of the concentration of Luegmi and $Na_2HPO_4$ quantity when fixing the time of incubation at 24 hours. Iso-response curve presented in Figure 3 proved the significant effect of these two compounds on the area of dispersion. Thus, a dispersion area of the order of 32.18 $cm^2$ and a ST decrease of about 56.1 % (26.34 mN/m) can be obtained by working with 26.23 % Luegmi and 0.1 % $Na_2HPO_4$.

To conclude, by analyzing the plots, the best biosurfactant corresponding of a ST reduction of about 55 % (ST of 27 mN/m) and an area of oil displacement of about 30 $cm^2$ (Diameter of 6 cm) can be achieved when inoculating 25 % Luegmi added with 0.1 % $Na_2HPO_4$ with an incubation time of 24 hours. The corresponding experiment was performed in four replicates, and our average value was obtained near to the predicted value described by the model analysis suggesting the effectiveness of the experimental and the response surface methodology in the optimization of the fermentation process. Thus, the application of experimental design together with the use of an economic nutrient sources permit to produce low-cost biosurfactants for potential use in bioremediation.

Generally, the success of the use of bio-surfactants depends on the costs of their production. However, the raw material has a significant impact on the final cost in most biotechnological processes (Singh et al. 2019). During this work, we were able to effectively produce biosurfactants using a food by-product “Luegmi”. This juice extracted from the heart of the date palm is harvested directly from a gargoulette placed on the top of the palm. The technique of recuperation is meticulous: prune the tree with a sharp tool, dig in the center to the tender part of the heart and collect the sap called «Luegmi». It is a very pleasant hygienic drink rich in sugars (about 12%) and minerals with a low content of nitrogen and fat. Sugars are mainly composed of sucrose, a simple sugar widely used for the production of lipopeptide biosurfactants by many strains (Kanna et al. 2014, Liang et al. 2017, Liu et al. 2012, Pereira et al. 2013, Singh et al. 2014). Thus, Luegmi can support the production of biosurfactants as substitutes for commercial sugars. This good production of lipopeptide of BI2 after a fermentation time of the order of 24 hours with the ease of recovery and purification of biosurfactants is of considerable interest and contributes to reduce considerably the cost of biosurfactant production. Generally, date palm generate numerous waste/by-products including pits, press-cake, cull dates, other residue, etc., date pits that pose disposal and environmental problem (Najib &Al-Yousef 2013). Their valorization to value added products present an interesting choice. They are used for the production of biofuels, biopolymers, biosurfactants, organic acids, antibiotics, industrial enzymes and are investigated as a functional food (Chandrasekaran &Bahkali 2013, Najib &Al-Yousef 2013). Date molasses was used by (Al-Bahry et al. 2013) and (Al-Dhabi et al. 2020) as a rich carbon source for biosurfactant production by *Bacillus subtilis* species. Moreover, previous studies described the production of biosurfactant on date syrup (Ghasemi et al. 2018, Kebbouche-Gana et al. 2013). Similarly, date juice was used previously as carbon source to produce Lactic acid by *Lactobacillus* species (Nancib et al. 2001, Yadav et al. 2011). In our study, it’s the first time to investigate the use of date juice as a novel carbon source for biosurfactant production. It permits to support a maximum biosurfactant production with a very low cost.

### *Biochemical characterization of B. mojavensis BI2 biosurfactant*

The keys of the use of biosurfactants are essentially the cost of their production and the ease of their recovery. In the first part of our paper, we have managed to achieve an economic and a high production yield of *B. mojavensis* BI2 derived biosurfactant. Then we tried to investigate the biochemical nature of the biosurfactant produced on Luegmi. In this context, we begin with an acid precipitation nearby pH of 2, then, we solubilize the pellet in a minimum of distilled water followed by increasing the pH to 8 (Mnif et al. 2013b, Mnif et al. 2013a). A second cycle of precipitation-dissolution was performed in order to eliminate the maximum of impurities. The crude extract obtained served to study the biosurfactant chemical nature and their functional properties.

### 1.1.1 TLC Analysis of the produced biosurfactant

The biochemical nature of the biosurfactant was confirmed by Thin Layer Chromatography Analysis after organic extraction. The crude extract obtained by twice acid precipitation-dissolution was extracted by a mixture of ethyl-acetate: methanol (8: 1) (v/v) (Coronel-León et al. 2015). TLC indicated the presence of a noteworthy spot, identified as a lipopeptide. In fact, they showed orange spot after spraying with ninhydrin (Sigma, St. Louis, MO) indicating the presence of amine groups; sugar observation with sulfuric acid-ethanol solution indicated the absence of carbohydrate compounds; however; iodine vapors indicated the presence of lipid moiety by the observation of yellow spot (Coronel-León et al. 2015). Also, ultraviolet observation of the TLC plate indicated the presence of red spots confirming the presence of amino-acids compounds. Results confirmed the biochemical identity of biosurfactants as lipopeptide compound. Results are similar to those obtained by (Balan et al. 2017, Coronel-León et al. 2015, Mnif et al. 2016b) describing the production of lipopeptide biosurfactant by *B. subtilis* SPB1, *B. licheniformis* and *Aneurinibacillus aneurinilyticus* SBP-11 respectively. Our results are in correlation with the first paper published reporting the production of lipopeptides compounds by *B. mojavensis* BI2. A further purification and spectrometric analysis may confirm and elucidate the nature of the different isoforms produced on Luegmi as a carbon and nitrogen sources.

### Ultraviolet spectra of BI2 derived biosurfactant

Generally, an Ultraviolet spectrum is a preliminary tool that permits to investigate the nature of the functional group constituting a bio-molecule. As observed in Fig. 4, absorption spectrum ranging from 200 to 600 nm wave-lengths showed the existence of 3 maximum of absorbance, one at 215 nm, the second at 230 nm and the third at 260 nm. The peak absorption at 230 nm is specific to the peptide bonds between the carboxyl functions carried by the amino acid α carbon and the amino functions carried by the next amino acid carbon in the peptide chain. The second peak at 260 nm is specific amino acid aromatic nuclei (Ghazala et al. 2019). These two peaks indicate the presence of a chain of amino acids or peptide part. However, peak absorption at 215 nm indicates the presence of a lipid. These results are similar to those published by (Ismail et al. 2013) and (Ghazala et al. 2019) suggested the lipopeptide nature of the biosurafctant.

### FTIR analysis

In addition to the UV spectrum, FTIR analysis can give an idea of the clusters present in the BI2 biosurfactant. Fig. 5 representing the IR spectrum of the biosurfactant produced by *Bacillus mojavensis* BI2 shows different functional groups characteristic of lipopeptide biosurfactants. A broad absorbance peak (centered around 3433 $cm^{-1}$) with wave numbers ranging from 3600 $cm^{-1}$ to 3100 $cm^{-1}$ was observed. It corresponds to C-H stretching vibrations and N-H stretching vibrations characteristic of compounds containing carbon with amino

groups corresponding to peptides (Anvari et al. 2015, Biniarz et al. 2017, Jemil et al. 2017, Pueyo et al. 2009, Varadavenkatesan &Murty 2013, Zhang et al. 2016). Moreover, a strong band was also around 1650 $cm^{-1}$ indicating the presence of a carbonyl group C=O (Habib et al. 2020, Pueyo et al. 2009, Varadavenkatesan &Murty 2013). Nevertheless, sharp absorbance peaks are observed at 1463 cm-1, 1379 cm-1, 2955 $cm^{-1}$ and 2854 $cm^{-1}$ and are indicative of aliphatic chains ($-CH_3$ and $-CH_2-$) in Figure 3 as described by (Jemil et al. 2017, Roy et al. 2013, Varadavenkatesan &Murty 2013, Zhang et al. 2016). Absorption at approximately 1400 cm-1 is also observed. It is characteristic of the aromatic groups as proposed by (Roy et al. 2013). However, there are also peaks at 1238 and 1118 $cm^{-1}$ that are likely due to C-O-C vibrations in esters as defined by (Habib et al. 2020). All these observations confirm the lipopeptide nature of the biosurfactant derived from *Bacillus mojavensis* BI2.

Generally, lipopeptides are amphiphilic compounds containing a polar hydrophilic peptide part composed of 7 amino acids (surfactin and iturin) or 10 amino acids (fengycin) linked to an apolar hydrophobic part. The latter is usually a single chain of fatty acid bound to the peptide by an amine bond for iturin or hydroxyl for surfactin and fengycin. The length of this chain varies from C12 to C16 for surfactin, from C14 to C17 for iturin and from C15 to C18 for fengycin (Mnif &Ghribi 2015b). Thus, lipopeptides are defined as set of isomers and isoforms differing in the nature and number of amino acids in the peptidic part and in the length of the fatty acid chain. Further purification by HPLC and identification by Mass Spectrum allows de define the different lipoepeptide isoforms produced by BI2.

### *Physic-chemical characterization of B. mojavensis BI2 biosurfactant*

#### Study of biosurfactant activity at different temperature, pH and salinities

**In the course of this work, we studied the effect different physic-chemical factors such as temperature, pH and sodium chloride concentration on biosurfactant activity by monitoring the surface tension reduction potential and the oil dispersant activity. They were evaluated using a crude lipopeptide preparation of BI2 derived biosurfactant at its Critical Micelle Concentration for TS measurement and 1000 mg/L for motor oil dispersion measurement.**

By varying the temperature, we observed an optimal decrease of the Surface Tension of water at temperatures ranging from 10°C to 30°C (Fig. 6). For higher temperature, surface activity gradually decreases to keep almost 52% of its relative activity at 80°C. For dispersant activity, the BI2 biosurfactant shows good thermo-activity as it retains more than 50% of its activity at temperatures below or equal to 80°C. These results are similar to those obtained by (Feng et al. 2019) and (Phulpoto et al. 2020). (Mnif et al. 2013b).

Regarding the pH activity, our results show that BI2 biosurfactant is active in a wide pH range as it retains almost 100% of its ST-decreasing power and more than 60% of its dispersing activity of motor oil at pH 4 to 10 (Fig. 7). However, for very acidic pH (2 and 3), there is a decrease in the different activities. This can be attributed to the physic-chemical properties of lipopeptide biosurfactants that precipitate at pH below 5 and are fully soluble at neutral and slightly alkaline pH (8 and 8.5) (Takashi et al. 2009). As a result, they can maintain good activity at pH 6-10 and can lose it at very low pH. Indeed, it can be said that BI2 biosurfactant has interesting activities at a wide range of pH; results similar to those published by, (Feng et al. 2019) and (Phulpoto et al. 2020) (Mnif et al. 2013b).

The study of BI2 biosurfactant activity at different salt concentration showed that sodium chloride addition increase considerably the Surface Tension reduction power for concentration higher than 0.5 % with a maximal activation of about 300 % in the presence of 1.5 to 2 % NaCl (Fig. 8). Moreover, it retains about 200 % of its residual activity at 3 % NaCl. So, we can assume that the sodium cation activate highly BI2 biosurfactant. The activating effect of sodium chloride and potassium chloride was also observed for *B. subtilis* derived surfactin (Thimon et al. 1992) and a *B. coagulans* derived biosurfactant (Huszcza &Burczyk 2003). As suggested by (Thimon et al. 1992), this could probably be explained by changes in the molecular area of biosurfactants. For the decreasing power of the TS, activity remains virtually stable with a very small decrease. Similarly, numerous studies reported the stability of surfactant activities of lipopeptide biosurfactants under saline conditions (Feng et al. 2019, Mnif et al. 2013b, Pathak &Keharia 2014, Phulpoto et al. 2020) (Mnif et al. 2013b). Notably, these interesting results offer the opportunity for BI2 biosurfactant to be used in the bioremediation of marine water contaminated by hydrocarbons.

### Study of BI2 biosurfactant stability after pre-incubation at different temperature, pH and salinities

The potential use of biosurfactants in various industrial processes involving thermal treatments requires a high degree of thermal stability to maintain their activities. In this framework, we studied the effect of thermal pre-incubation of BI2 lipopeptide on its surface activities. Results presented in Fig. 9 suggested a perfect thermostability of our biosurfactant. In fact, it retains about 100% of its Surface Tension reducing power and over 90% of its oil dispersant activity of oil in the temperature range studied. In particular, there is an improvement in the dispersant activity of the oil for heat treatments ranging from 60 to 90°C. Similarly, numerous previous studies reported the stability of the surface activity of lipopeptide biosurfactants (Chen et al. 2017, Feng et al. 2019, Ghazala et al. 2017, Kiran et al. 2017, Martins et al. 2018, Purwasena et al. 2019, Qiao &Shao 2010, Zouari et al. 2019b). As observed in Fig. 10, one-hour pre-incubation of the BI2 biosurfactant at different pH values ranging from 2 to 10 shows that it retains more than 80% of its Surface Tension decreasing power at pH values ranging from 3 to 10. These results may be in favor of broadening the scope of application of BI2 lipopeptide. They are similar to numerous previous reports (Chen et al. 2017, Feng et al. 2019, Ghazala et al. 2017, Kiran et al. 2017, Martins et al. 2018, Purwasena et al. 2019, Qiao &Shao 2010, Zouari et al. 2019b). However, the dispersant activity of the oil is strongly attenuated at a strongly acidic pH ranging from 2 to 4. It can be explained by the precipitation of biosurfactant by the effect of acidity.

It is necessary to study the effect of salinity on the stability of biosurfactant to investigate its application in the bioremediation of hydrocarbon-contaminated seawater. Thus, the biosurfactant of BI2 shows perfect stability after pre-incubations in the presence of high concentrations of NaCl confirmed by measurements of its two surface activities (Fig. 11). However, a slight alteration in its Surface Tension decreasing power is observed after pre-incubation in the presence of 5% NaCl. For the dispersant activity of motor oil, slight activations are observed at concentrations ranging from 0.5 to 4% NaCl. These results are similar to numerous previous work reporting the stability of the surface activities of biological surfactants after pre-incubation in the presence of decreasing NaCl concentration (Chen et al. 2017, Feng et al. 2019, Ghazala et al. 2017, Kiran et al. 2017, Martins et al. 2018, Purwasena et al. 2019, Qiao &Shao 2010, Zouari et al. 2019b). This offers a broad spectrum of application of BI2 biosurfactant in the bioremediation of saline and sea waters contaminated by hydrocarbons.

Compared with chemical surfactants, usually inhibited by high NaCl concentration; by thermal treatments and extreme pH values; BI2 derived biosurfactant can represents an interesting candidate for the bioremediation of sea waters contaminated by oil spill.

# Conclusion

In the present investigation, a naturally potential biosurfactant producer bacterium *Bacillus mojavensis* BI2 (MW130250) was isolated from a Tunisian soil (first report). It produces a biosurfactant with interesting surface tension reducing power and effective oil dispersing capacity. Having great interest for potential application in environmental field and bioremediation, we tried to optimize an economic production of *B. mojavensis* BI2 derived lipopeptide on the date juice called “Luegmi”; a food by-product extracted from date palm very rich in sugar and mineral elements. Production optimization by the application of a Box-Bhenken design and response surface methodology permit to reach a surface tension reduction oil dispersing capacities of 55 % (ST of 27 mN/m) and 30 $cm^2$ (Diameter of 6 cm) respectively after 24 hours of incubation. After production optimization, we characterize the biosurfactant by UV-Spectra and TLC. It showed a lipopeptide nature. In order to be applied in environmental field, we studied the effect of different physic-chemical factors on biosurfactant activity and stability. Results showed an interesting thermal and pH activity. Moreover, in the presence of increasing concentration of salts, lipopeptide derived from *B. mojavensis* BI2 maintain their surface activities in addition to a slight activation of the oil dispersion capacity. Therefore, the economic production of *B. mojavensis* derived lipopeptides in addition to their strong stability over wide range of environmental factors permits their application in various fields.

# Abbreviations

ZNI5 BioS: Biosurfactant produced by *Bacillus subtilis* ZNI5.

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

All authors read the final manuscript and approved its submission to Bioresources and Bioprocessing.

### Availability of data and materials

The data sets supporting the conclusions of this article are included in the article.

### Funding

Funding for this research work was granted by the Ministry of Higher Education and Research of Tunisia.

### Authors’ contributions

All authors directly participated in the planning, execution, or analysis of this study. All authors read and approved the final manuscript.

### Acknowledgements

This work has been supported by grants from the Tunisian Ministry of Higher Education, Scientific Research and Technology. It is a part of a research project on biosurfactant production, characterization and application.

### Competing interest

The authors declare that they have no competing interests.

### Author information

**Inès Mnif, Amir Bouallegue, Salwa Mekki, Dhouha Ghribi**

The first Author Inès Mnif is an Assistant Teacher in the Faculty of Science of Gabes, University of Gabes, Tunisia and Member of the Laboratory of Biochemistry and Enzymatic Engineering and The Laboratory of Plants Enhancement and Agro-ressources Valorization of the National School of Engineering of Sfax, Tunisia

The Second Author Amir Bouallegue is a Doctor Studuent and Member of the Laboratory of Plants Enhancement and Agro-ressources Valorization of the National School of Engineering of Sfax, Tunisia

The Third Author Salwa Mekki is a Doctor Studient and Member of the Laboratory of Plants Enhancement and Agro-ressources Valorization of the National School of Engineering of Sfax, Tunisia

The Fourth Author Dhouha Ghribi is a Professor in the Higher Institute of Biotechnology of Sfax, Tunisia and Member of the Laboratory of Plants Enhancement and Agro-ressources Valorization of the National School of Engineering of Sfax, Tunisia

### Publisher's Note



## References

Abushady H, Bashandy A, Aziz N, Ibrahim H (2005): Molecular characterization of Bacillus subtilis surfactin producing strain and the factors affecting its production. International Journal of Agriculture and Biology 3, 337-344

Al-Bahry S, Al-Wahaibi Y, Elshafie A, Al-Bemani A, Joshi S, Al-Makhmari H, Al-Sulaimani H (2013): Biosurfactant production by Bacillus subtilis B20 using date molasses and its possible application in enhanced oil recovery. International Biodeterioration & Biodegradation 81, 141-146

Al-Dhabi NA, Esmail GA, Valan Arasu M (2020): Enhanced Production of Biosurfactant from Bacillus subtilis Strain Al-Dhabi-130 under Solid-State Fermentation Using Date Molasses from Saudi Arabia for Bioremediation of Crude-Oil-Contaminated Soils. International Journal of Environmental Research and Public Health 17, 8446

Anvari S, Hajfarajollah H, Mokhtarani B, Noghabi KA (2015): Physiochemical and thermodynamic characterization of lipopeptide biosurfactant secreted by Bacillus tequilensis HK01. RSC advances 5, 91836-91845

Balan SS, Kumar CG, Jayalakshmi S (2017): Aneurinifactin, a new lipopeptide biosurfactant produced by a marine Aneurinibacillus aneurinilyticus SBP-11 isolated from Gulf of Mannar: Purification, characterization and its biological evaluation. Microbiological research 194, 1-9

Bartal A, Vigneshwari A, Bóka B, Vörös M, Takács I, Kredics L, Manczinger L, Varga M, Vágvölgyi C, Szekeres A (2018): Effects of different cultivation parameters on the production of surfactin variants by a Bacillus subtilis strain. Molecules 23, 2675

Beltran-Gracia E, Macedo-Raygoza G, Villafaña-Rojas J, Martinez-Rodriguez A, Chavez-Castrillon YY, Espinosa-Escalante FM, Di Mascio P, Ogura T, Beltran-Garcia MJ (2017): Production of lipopeptides by fermentation processes: Endophytic bacteria, fermentation strategies and easy methods for bacterial selection. Fermentation processes, 199-222

Bertrand B, Martínez-Morales F, Rosas-Galván NS, Morales-Guzmán D, Trejo-Hernández MR (2018): Statistical design, a powerful tool for optimizing biosurfactant production: a review. Colloids and Interfaces 2, 36

Biniarz P, Łukaszewicz M, Janek T (2017): Screening concepts, characterization and structural analysis of microbial-derived bioactive lipopeptides: a review. Critical reviews in biotechnology 37, 393-410

Chandrasekaran M, Bahkali AH (2013): Valorization of date palm (Phoenix dactylifera) fruit processing by-products and wastes using bioprocess technology–Review. Saudi journal of biological sciences 20, 105-120

Chen Y, Liu S, Mou H, Ma Y, Li M, Hu X (2017): Characterization of lipopeptide biosurfactants produced by Bacillus licheniformis MB01 from marine sediments. Front. Microbiol. 2017, V. 8

Cho KM, Math RK, Hong SY, Islam SMA, Mandanna DK, Cho JJ, Yun MG, Kim JM, Yun HD (2009): Iturin produced by Bacillus pumilus HY1 from Korean soybean sauce (kanjang) inhibits growth of aflatoxin producing fungi. Food Control 20, 402-406

Chooklin CS, Phertmean S, Cheirsilp B, Maneerat S, Saimmai A (2013): Utilization of palm oil mill effluent as a novel and promising substrate for biosurfactant production by Nevskia ramosa NA3. Songklanakarin Journal of Science & Technology 35

Coronel-León J, de Grau G, Grau-Campistany A, Farfan M, Rabanal F, Manresa A, Marqués AM (2015): Biosurfactant production by AL 1.1, a Bacillus licheniformis strain isolated from Antarctica: production, chemical characterization and properties. Annals of microbiology 65, 2065-2078

Dang Y, Zhao F, Liu X, Fan X, Huang R, Gao W, Wang S, Yang C (2019): Enhanced production of antifungal lipopeptide iturin A by Bacillus amyloliquefaciens LL3 through metabolic engineering and culture conditions optimization. Microbial cell factories 18, 1-14

Eswari JS, Anand M, Venkateswarlu C (2016): Optimum culture medium composition for lipopeptide production by Bacillus subtilis using response surface model-based ant colony optimization. Sadhana 41, 55-65

Feng J-Q, Gang H-Z, Li D-S, Liu J-F, Yang S-Z, Mu B-Z (2019): Characterization of biosurfactant lipopeptide and its performance evaluation for oil-spill remediation. RSC advances 9, 9629-9632

Ghasemi A, Moosavi-Nasab M, Behzadnia A, Rezaei M (2018): Enhanced biosurfactant production with low-quality date syrup by Lactobacillus rhamnosus using a fed-batch fermentation. Food science and biotechnology 27, 1137-1144

Ghazala I, Bouassida M, Krichen F, Manuel Benito J, Ellouz-Chaabouni S, Haddar A (2017): Anionic lipopeptides from Bacillus mojavensis I4 as effective antihypertensive agents: Production, characterization, and identification. Engineering in life sciences 17, 1244-1253

Ghazala I, Bouallegue A, Haddar A, Ellouz-Chaabouni S (2019): Characterization and production optimization of biosurfactants by Bacillus mojavensis I4 with biotechnological potential for microbial enhanced oil recovery. Biodegradation 30, 235-245

Ghribi D, Abdelkefi-Mesrati L, Mnif I, Kammoun R, Ayadi I, Saadaoui I, Maktouf S, Chaabouni-Ellouze S (2012): Investigation of antimicrobial activity and statistical optimization of Bacillus subtilis SPB1 biosurfactant production in solid-state fermentation. Journal of Biomedicine and Biotechnology 2012

Habib S, Ahmad SA, Wan Johari WL, Abd Shukor MY, Alias SA, Smykla J, Saruni NH, Abdul Razak NS, Yasid NA (2020): Production of lipopeptide biosurfactant by a hydrocarbon-degrading Antarctic Rhodococcus. International journal of molecular sciences 21, 6138

Hippolyte MT, Augustin M, Hervé TM, Robert N, Devappa S (2018): Application of response surface methodology to improve the production of antimicrobial biosurfactants by Lactobacillus paracasei subsp. tolerans N2 using sugar cane molasses as substrate. Bioresources and Bioprocessing 5, 1-16

Hmidet N, Ben Ayed H, Jacques P, Nasri M (2017): Enhancement of surfactin and fengycin production by Bacillus mojavensis A21: application for diesel biodegradation. BioMed research international 2017

Hu F, Liu Y, Li S (2019): Rational strain improvement for surfactin production: enhancing the yield and generating novel structures. Microbial cell factories 18, 1-13

Huszcza E, Burczyk B (2003): Biosurfactant production by Bacillus coagulans. Journal of Surfactants and Detergents 6, 61-64

Ismail W, Al-Rowaihi IS, Al-Humam AA, Hamza RY, El Nayal AM, Bououdina M (2013): Characterization of a lipopeptide biosurfactant produced by a crude-oil-emulsifying Bacillus sp. I-15. International Biodeterioration &

Biodegradation 84, 168-178

Jemil N, Ayed HB, Manresa A, Nasri M, Hmidet N (2017): Antioxidant properties, antimicrobial and anti-adhesive activities of DCS1 lipopeptides from Bacillus methylotrophicus DCS1. BMC microbiology 17, 1-11

Joshi S, Bharucha C, Jha S, Yadav S, Nerurkar A, Desai AJ (2008): Biosurfactant production using molasses and whey under thermophilic conditions. Bioresource technology 99, 195-199

Joy S, Rahman PK, Sharma S (2017): Biosurfactant production and concomitant hydrocarbon degradation potentials of bacteria isolated from extreme and hydrocarbon contaminated environments. Chemical Engineering Journal 317, 232-241

Kanna R, Gummadi SN, Kumar GS (2014): Production and characterization of biosurfactant by Pseudomonas putida MTCC 2467. Journal of Biological Sciences 14, 436

Kebbouche-Gana S, Gana ML, Ferrioune I, Khemili S, Lenchi N, Akmouci-Toumi S, Bouanane-Darenfed NA, Djelali N-E (2013): Production of biosurfactant on crude date syrup under saline conditions by entrapped cells of Natrialba sp. strain E21, an extremely halophilic bacterium isolated from a solar saltern (Ain Salah, Algeria). Extremophiles 17, 981-993

Kim KM, Lee JY, Kim CK, Kang JS (2009): Isolation and characterization of surfactin produced by Bacillus polyfermenticus KJS-2. Archives of pharmacal research 32, 711-715

Kiran GS, Priyadharsini S, Sajayan A, Priyadharsini GB, Poulose N, Selvin J (2017): Production of lipopeptide biosurfactant by a marine Nesterenkonia sp. and its application in food industry. Frontiers in microbiology 8, 1138

Liang X, Shi R, Radosevich M, Zhao F, Zhang Y, Han S, Zhang Y (2017): Anaerobic lipopeptide biosurfactant production by an engineered bacterial strain for in situ microbial enhanced oil recovery. RSC advances 7, 20667-20676

Lin H-Y, Rao YK, Wu W-S, Tzeng Y-M (2007): Ferrous ion enhanced lipopeptide antibiotic iturin A production from Bacillus amyloliquefaciens B128. International Journal of Applied Science and Engineering 5, 123-132

Liu X, Ren B, Gao H, Liu M, Dai H, Song F, Yu Z, Wang S, Hu J, Kokare CR (2012): Optimization for the production of surfactin with a new synergistic antifungal activity. PloS one 7, e34430

Luna JM, Rufino RD, Jara AMA, Brasileiro PP, Sarubbo LA (2015): Environmental applications of the biosurfactant produced by Candida sphaerica cultivated in low-cost substrates. Colloids and Surfaces A: Physicochemical and Engineering Aspects 480, 413-418

Mani P, Sivakumar P, Balan SS (2016): Economic production and oil recovery efficiency of a lipopeptide biosurfactant from a novel marine bacterium Bacillus simplex. Achievements in the Life Sciences 10, 102-110

Martins PC, Bastos CG, Granjeiro PA, Martins VG (2018): New lipopeptide produced by Corynebacterium aquaticum from a low-cost substrate. Bioprocess and biosystems engineering 41, 1177-1183

Mnif I, Sahnoun R, Ellouze-Chaabouni S, Ghribi D (2013a): Evaluation of B. subtilis SPB1 biosurfactants' potency for diesel-contaminated soil washing: optimization of oil desorption using Taguchi design. Environmental Science and Pollution Research 21, 851-861

Mnif I, Ellouze-Chaabouni S, Ghribi D (2013b): Economic production of Bacillus subtilis SPB1 biosurfactant using local agro-industrial wastes and its application in enhancing solubility of diesel. Journal of Chemical Technology & Biotechnology 88, 779-787

Mnif I, Elleuch M, Chaabouni SE, Ghribi D (2013c): Bacillus subtilis SPB1 biosurfactant: production optimization and insecticidal activity against the carob moth Ectomyelois ceratoniae. Crop Protection 50, 66-72

Mnif I, Besbes S, Ellouze-Ghorbel R, Ellouze-Chaabouni S, Ghribi D (2013d): Improvement of bread dough quality by Bacillus subtilis SPB1 biosurfactant addition: optimized extraction using response surface methodology. Journal of the Science of Food and Agriculture 93, 3055-3064

Mnif I, Ellouze-Chaabouni S, Ayedi Y, Ghribi D (2014): Treatment of diesel-and kerosene-contaminated water by B. subtilis SPB1 biosurfactant-producing strain. Water Environment Research 86, 707-716

Mnif I, Ghribi D (2015a-a): Microbial derived surface active compounds: properties and screening concept. World Journal of Microbiology and Biotechnology 31, 1001-1020

Mnif I, Ghribi D (2015a-b): Microbial derived surface active compounds: properties and screening concept. World Journal of Microbiology Biotechnology 31, 1001-1020

Mnif I, Mnif S, Sahnoun R, Maktouf S, Ayedi Y, Ellouze-Chaabouni S, Ghribi D (2015a): Biodegradation of diesel oil by a novel microbial consortium: comparison between co-inoculation with biosurfactant-producing strain and exogenously added biosurfactants. Environmental Science and Pollution Research 22, 14852-14861

Mnif I, Fendri R, Ghribi D (2015b): Malachite green bioremoval by a newly isolated strain Citrobacter sedlakii RI11; enhancement of the treatment by biosurfactant addition. Water Science and Technology 72, 1283-1293

Mnif I, Ghribi D (2015b): Lipopeptide surfactants: production, recovery and pore forming capacity. Peptides 71, 100-112

Mnif I, Fendri R, Ghribi D (2015c): Biosorption of Congo Red from aqueous solution by Bacillus weihenstephanensis RI12; effect of SPB1 biosurfactant addition on biodecolorization potency. Water Science and Technology 72, 865-874

Mnif I, Ghribi D (2015c): Review lipopeptides biosurfactants: mean classes and new insights for industrial, biomedical, and environmental applications. Peptide Science 104, 129-147

Mnif I, Maktouf S, Fendri R, Kriaa M, Ellouze S, Ghribi D (2016a): Improvement of methyl orange dye biotreatment by a novel isolated strain, Aeromonas veronii GRI, by SPB1 biosurfactant addition. Environmental Science and Pollution Research 23, 1742-1754

Mnif I, Grau-Campistany A, Coronel-León J, Hammami I, Triki MA, Manresa A, Ghribi D (2016b): Purification and identification of Bacillus subtilis SPB1 lipopeptide biosurfactant exhibiting antifungal activity against Rhizoctonia bataticola and Rhizoctonia solani. Environmental Science and Pollution Research 23, 6690-6699

Moshtagh B, Hawboldt K, Zhang B (2019): Optimization of biosurfactant production by Bacillus subtilis N3-1P using the brewery waste as the carbon source. Environmental technology 40, 3371-3380

Mukherjee S, Das P, Sivapathasekaran C, Sen R (2009): Antimicrobial biosurfactants from marine Bacillus circulans: extracellular synthesis and purification. Letters in applied microbiology 48, 281-288

Najib H, Al-Yousef YM (2013): Value-Added Utilization of Dates By-Products. Dates: Postharvest Science, Processing Technology and Health Benefits, 233-260

Nancib N, Nancib A, Boudjelal A, Benslimane C, Blanchard F, Boudrant J (2001): The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by Lactobacillus casei subsp. rhamnosus. Bioresource Technology 78, 149-153

Ndlovu T, Rautenbach M, Khan S, Khan W (2017): Variants of lipopeptides and glycolipids produced by Bacillus amyloliquefaciens and Pseudomonas aeruginosa cultured in different carbon substrates. AMB Express 7, 1-13

Nurfarahin AH, Mohamed MS, Phang LY (2018): Culture medium development for microbial-derived surfactants production—an overview. Molecules 23, 1049

Ohadi M, Dehghannoudeh G, Shakibaie M, Banat IM, Pournamdari M, Forootanfar H (2017): Isolation, characterization, and optimization of biosurfactant production by an oil-degrading Acinetobacter junii B6 isolated from an Iranian oil excavation site. Biocatalysis and Agricultural Biotechnology 12, 1-9

Olasanmi IO, Thring RW (2018): The role of biosurfactants in the continued drive for environmental sustainability. Sustainability 10, 4817

Pacwa-Płociniczak M, Płaza GA, Piotrowska-Seget Z, Cameotra SS (2011): Environmental applications of biosurfactants: recent advances. International Journal of Molecular Sciences 12, 633-654

Paraszkiewicz K, Bernat P, Kuśmierska A, Chojniak J, Płaza G (2018): Structural identification of lipopeptide biosurfactants produced by Bacillus subtilis strains grown on the media obtained from renewable natural resources. Journal of Environmental Management 209, 65-70

Pathak KV, Keharia H (2014): Application of extracellular lipopeptide biosurfactant produced by endophytic Bacillus subtilis K1 isolated from aerial roots of banyan (Ficus benghalensis) in microbially enhanced oil recovery (MEOR). 3 Biotech 4, 41-48

Patowary K, Patowary R, Kalita MC, Deka S (2017): Characterization of biosurfactant produced during degradation of hydrocarbons using crude oil as sole source of carbon. Frontiers in microbiology 8, 279

Pereira JF, Gudiña EJ, Costa R, Vitorino R, Teixeira JA, Coutinho JA, Rodrigues LR (2013): Optimization and characterization of biosurfactant production by Bacillus subtilis isolates towards microbial enhanced oil

recovery applications. Fuel 111, 259-268

Phulpoto IA, Yu Z, Hu B, Wang Y, Ndayisenga F, Li J, Liang H, Qazi MA (2020): Production and characterization of surfactin-like biosurfactant produced by novel strain Bacillus nealsonii S2MT and it's potential for oil contaminated soil remediation. Microbial cell factories 19, 1-12

Pueyo MT, Bloch C, Carmona-Ribeiro AM, Di Mascio P (2009): Lipopeptides produced by a soil Bacillus megaterium strain. Microbial ecology 57, 367

Purwasena IA, Astuti DI, Syukron M, Amaniyah M, Sugai Y (2019): Stability test of biosurfactant produced by Bacillus licheniformis DS1 using experimental design and its application for MEOR. Journal of Petroleum Science and Engineering 183, 106383

Qiao N, Shao Z (2010): Isolation and characterization of a novel biosurfactant produced by hydrocarbon-degrading bacterium Alcanivorax dieselolei B-5. Journal of applied microbiology 108, 1207-1216

Ramírez IM, Tsaousi K, Rudden M, Marchant R, Alameda EJ, Román MG, Banat IM (2015): Rhamnolipid and surfactin production from olive oil mill waste as sole carbon source. Bioresource technology 198, 231-236

Rodrigues L, Teixeira J, Oliveira R (2006): Low-cost fermentative medium for biosurfactant production by probiotic bacteria. Biochemical Engineering Journal 32, 135-142

Roy A, Mahata D, Paul D, Korpole S, Franco OL, Mandal SM (2013): Purification, biochemical characterization and self-assembled structure of a fengycin-like antifungal peptide from Bacillus thuringiensis strain SM1. Frontiers in microbiology 4, 332

Sarubbo LA, Luna J, Rufino RD, Brasileiro P (2016): Production of a Low-cost Biosurfactant for Application in the Remediation of Sea water Contaminated with Petroleum Derivates. Chemical Engineering Transactions 49, 523-528

Shi J, Zhu X, Lu Y, Zhao H, Lu F, Lu Z (2018): Improving iturin A production of Bacillus amyloliquefaciens by genome shuffling and its inhibition against Saccharomyces cerevisiae in orange juice. Frontiers in microbiology 9, 2683

Shih L, Kuo C-Y, Hsieh F-C, Kao S-S, Hsieh C (2008): Use of surface response methodology to optimize culture conditions for iturin A production by Bacillus subtilis in solid-state fermentation. Journal of the Chinese Institute of Chemical Engineers 39, 635-643

Singh AK, Rautela R, Cameotra SS (2014): Substrate dependent in vitro antifungal activity of Bacillus sp strain AR2. Microbial cell factories 13, 1-11

Singh P, Patil Y, Rale V (2019): Biosurfactant production: emerging trends and promising strategies. Journal of applied microbiology 126, 2-13

Soares CC, de Andrade ASA, Ferreira GFD, de Almeida AF, Druzian JI, Lobato AKdCL (2018): Biosurfactant production by Bacillus subtilis UFPEDA 86 using papaya (Carica papaya L.) waste as substrate: Viability

studies and pH influence of the culture medium. African Journal of Biotechnology 17, 928-937

Takashi A, Jin GY, Mizumoto S, Shahedur RM, Okuno K, Shoda M (2009): Solid state fermentation of lipopeptide antibiotic iturin A by using a novel solid state fermentation reactor system. Journal of Environmental Sciences 21, S162-S165

Thimon L, Peypoux F, Michel G (1992): Interactions of surfactin, a biosurfactant from Bacillus subtilis, with inorganic cations. Biotechnology letters 14, 713-718

Varadavenkatesan T, Murty VR (2013): Production of a lipopeptide biosurfactant by a novel Bacillus sp. and its applicability to enhanced oil recovery. International Scholarly Research Notices 2013

Wang Q, Chen S, Zhang J, Sun M, Liu Z, Yu Z (2008): Co-producing lipopeptides and poly-γ-glutamic acid by solid-state fermentation of Bacillus subtilis using soybean and sweet potato residues and its biocontrol and fertilizer synergistic effects. Bioresource technology 99, 3318-3323

Wei Y-H, Chu I-M (2002): Mn 2+ improves surfactin production by Bacillus subtilis. Biotechnology Letters 24, 479-482

Wei YH, Wang LF, Chang JS (2004): Optimizing iron supplement strategies for enhanced surfactin production with Bacillus subtilis. Biotechnology Progress 20, 979-983

Yadav AK, Bipinraj NK, Chaudhari AB, Kothari RM (2011): Production of L (+) lactic acid from sweet sorghum, date palm, and golden syrup as alternative carbon sources. Starch-Stärke 63, 632-636

Zhang J, Xue Q, Gao H, Lai H, Wang P (2016): Production of lipopeptide biosurfactants by Bacillus atrophaeus 5-2a and their potential use in microbial enhanced oil recovery. Microbial cell factories 15, 1-11

Zouari O, Lecouturier D, Rochex A, Chataigne G, Dhulster P, Jacques P, Ghribi D (2019b): Bio-emulsifying and biodegradation activities of syringafactin producing Pseudomonas spp. strains isolated from oil contaminated soils. Biodegradation 30, 259-272

Zouari R, Ellouze-Chaabouni S, Ghribi-Aydi D (2014): Optimization of Bacillus subtilis SPB1 biosurfactant production under solid-state fermentation using by-products of a traditional olive mill factory. Achievements in the Life Sciences 8, 162-169

Zouari R, Ellouze-Chaabouni S, Ghribi D (2019a): Use of Butter Milk and Poultry-Transforming Wastes for Enhanced Production of Bacillus subtilis SPB1 Biosurfactant in Submerged Fermentation. Journal of Microbiology, Biotechnology and Food Sciences 2019b, 462-466

# Tables

**Table 1:** Box-Bhenken design and observed and predicted results for surface tension decrease and oil displacement area

| Exp N° | $X_1$ (Luegmi %) | $X_2$ ($Na_2HPO_4$ quantity %) | $X_3$ (Fermenta-tion time h) | Y1 : Surface Tension Decrease (%) | | Y2 : Oil displacement area ($cm^2$) | |
|---|---|---|---|---|---|---|---|
| | | | | Observed | Predicted | Observed | Predicted |
| 1 | -1 (10) | -1 (0.02) | 0 (36) | 41.890 | 37.316 | 1.760 | 1.484 |
| 2 | 1 (30) | -1 (0.02) | 0 (36) | 26.890 | 28.404 | 0.190 | -1.846 |
| 3 | -1 (10) | 1 (0.1) | 0 (36) | 46.200 | 44.686 | 7.060 | 9.096 |
| 4 | 1 (30) | 1 (0.1) | 0 (36) | 54.130 | 58.704 | 32.150 | 32.426 |
| 5 | -1 (10) | 0 (0.06) | -1 (24) | 42.750 | 45.487 | 3.140 | 1.106 |
| 6 | 1 (30) | 0 (0.06) | -1 (24) | 54.860 | 51.510 | 19.620 | 19.346 |
| 7 | -1 (10) | 0 (0.06) | 1 (48) | 44.480 | 47.830 | 7.060 | 7.334 |
| 8 | 1 (30) | 0 (0.06) | 1 (48) | 49.650 | 46.912 | 7.060 | 9.094 |
| 9 | 0 (20) | -1 (0.02) | -1 (24) | 26.200 | 28.036 | 0.190 | 2.500 |
| 10 | 0 (20) | 1 (0.1) | -1 (24) | 51.030 | 49.806 | 23.740 | 23.737 |
| 11 | 0 (20) | -1 (0.02) | 1 (48) | 28.620 | 29.844 | 0.780 | 0.782 |
| 12 | 0 (20) | 1 (0.1) | 1 (48) | 47.580 | 45.744 | 23.740 | 21.430 |
| 13 | 0 (20) | 0 (0.06) | 0 (36) | 47.580 | 47.495 | 19.620 | 14.420 |
| 14 | 0 (20) | 0 (0.06) | 0 (36) | 46.550 | 47.495 | 9.610 | 14.420 |
| 15 | 0 (20) | 0 (0.06) | 0 (36) | 46.890 | 47.495 | 12.560 | 14.420 |
| 16 | 0 (20) | 0 (0.06) | 0 (36) | 48.960 | 47.495 | 15.890 | 14.420 |

**Table 2:** Estimated effect, regression coefficient and corresponding t and p values for biosurfactant production

| Noun | Coefficient | | F. Inflation | | Ecart-Type | | t. exp | | Signifiance % | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Y1 | Y2 | Y1 | Y2 | Y1 | Y2 | Y1 | Y2 | Y1 | Y2 |
| $b_0$ | 47.495 | 14.420 | | | 0.533 | 1.863 | 89.06 | 7.74 | <0.01*** | 0.0245*** |
| $b_1$ | 1.276 | 5 | 1 | 1 | 0.377 | 1.318 | 3.38 | 3.79 | 4.29* | 0.0902 |
| $b_2$ | 9.418 | 10.471 | 1 | 1 | 0.377 | 1.318 | 24.97 | 7.95 | 0.0141*** | 0.0211*** |
| $b_3$ | -0.564 | -1.006 | 1 | 1 | 0.377 | 1.318 | -1.50 | -0.76 | 23.2 | 47.4 |
| $b_{1-1}$ | 2.180 | -3.511 | 1 | 1 | 0.533 | 1.863 | 4.09 | -1.88 | 2.65* | 10.9 |
| $b_{2-2}$ | -7.398 | -0.619 | 1 | 1 | 0.533 | 1.863 | -13.87 | -0.33 | 0.0811*** | 75.1 |
| $b_{3-3}$ | -1.740 | -1.689 | 1 | 1 | 0.533 | 1.863 | -3.26 | -0.91 | 4.70* | 40 |
| $b_{1-2}$ | 5.732 | 6.665 | 1 | 1 | 0.533 | 1.863 | 10.75 | 3.58 | 0.172** | 1.17* |
| $b_{1-3}$ | -1.735 | -4.120 | 1 | 1 | 0.533 | 1.863 | -3.25 | -2.21 | 4.74* | 6.9 |
| $b_{2-3}$ | -1.468 | -0.147 | 1 | 1 | 0.533 | 1.863 | -2.75 | -0.08 | 7.1 | 93.9 |

(***): significant at the level 99.99 %

(**): significant at the level 99.9 %

(*): significant at the level 99 %

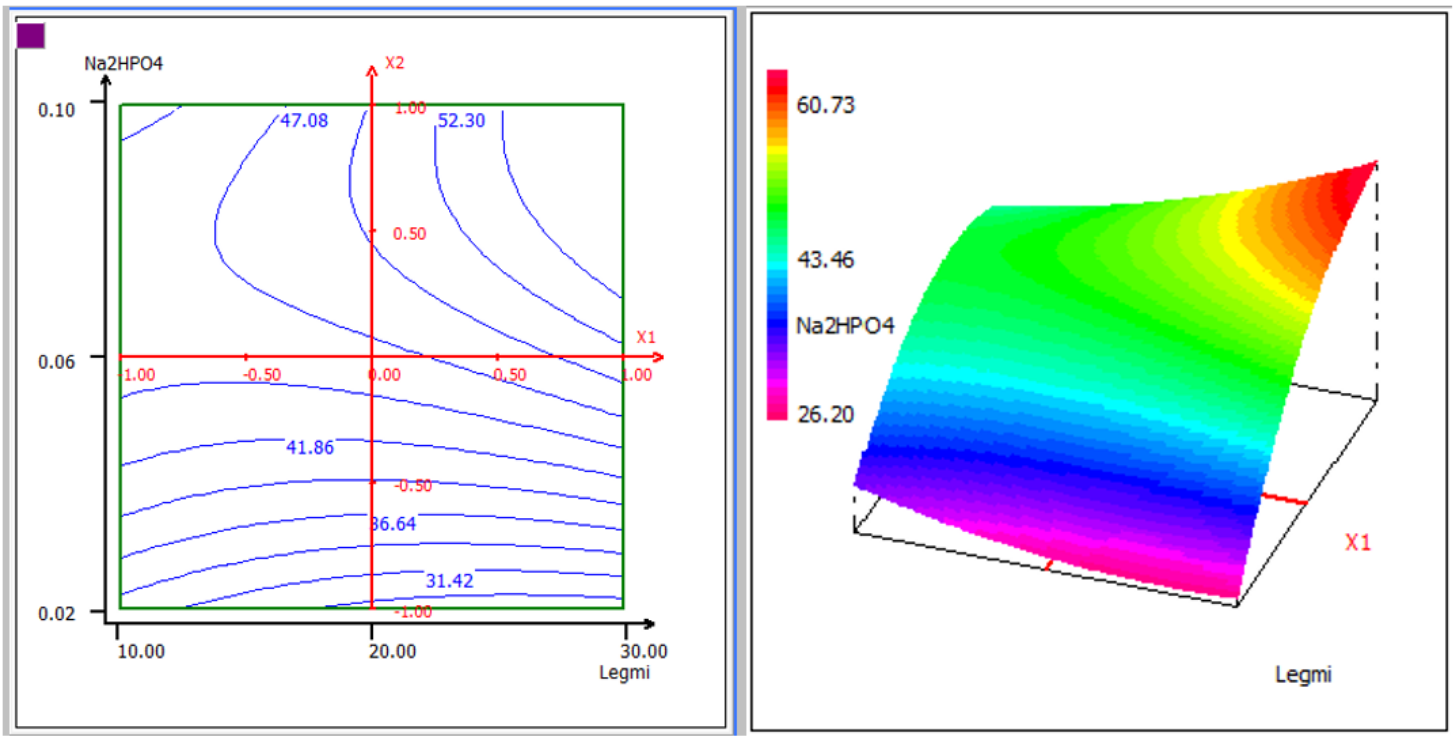
NS: Non Significant

**Table 3:** ANOVA Analysis for biosurfactant production

| Source of Variation | Sum of squares | | Degree of freedom | Mean square | | F-value | | Significance (Pr>F) | |
|---|---|---|---|---|---|---|---|---|---|
| | Y1 | Y2 | | Y1 | Y2 | Y1 | Y2 | Y1 | Y2 |
| Regression | 1127.2 | 1.3932 | 9 | 5.6674 | 1.5480 | 15.983 | 11.145 | 0.155** | 0.415** |
| Residual | 97 | 8.3336 | 6 | 0.3546 | 1.3889 | | | | |
| Lac of fit | 93.59 | 2.750 | 3 | 0.2925 | 9.1799 | 0.702 | 0.494 | 61.1 | 71.2 |
| Pure Error | 3.4 | 5.5797 | 3 | 0.4167 | 1.8599 | | | | |
| Total | 1224.2 | 1.4765 | 15 | | | | | | |

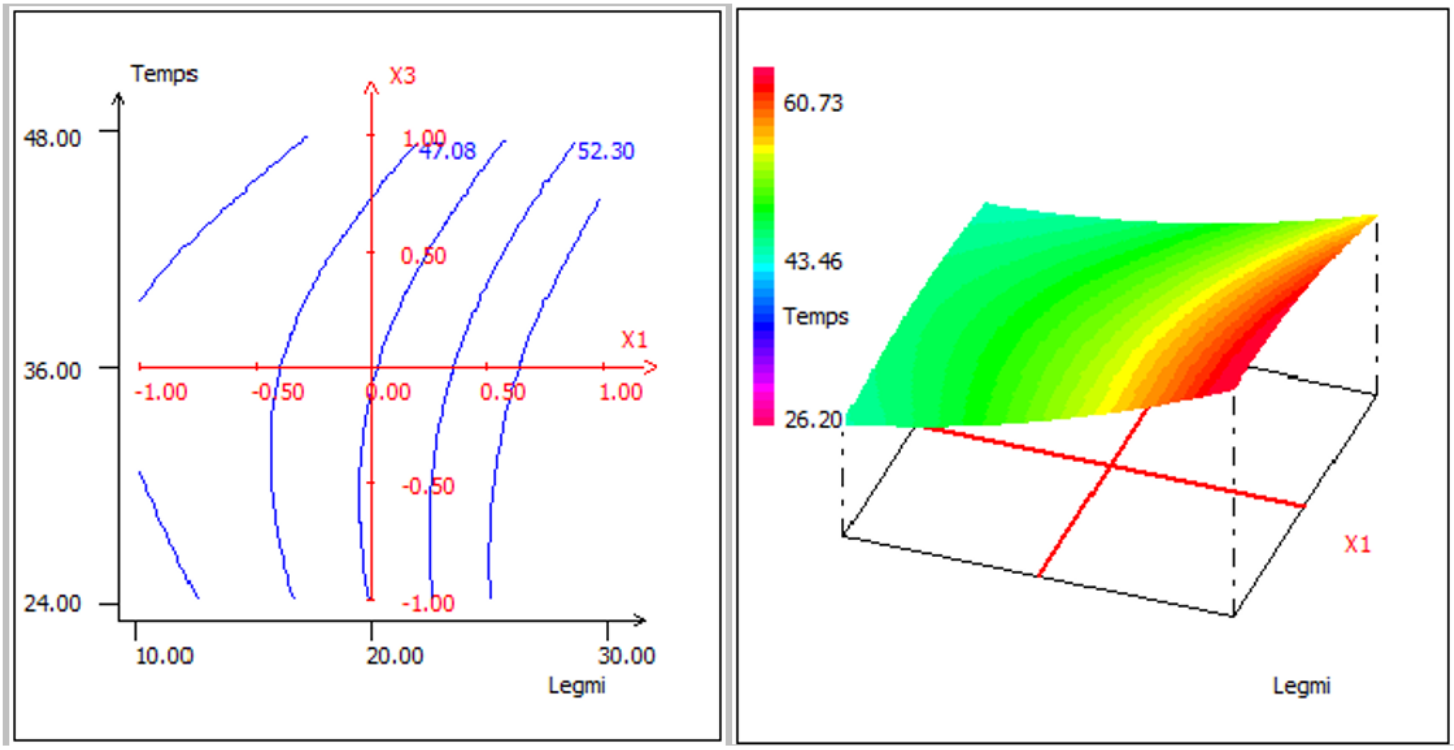
(**): significant at the level 99.9 %

# Figures




## Figure 1

Pattern of BI2 lipopeptide biosurfactant production visualized by surface tension decreasing power: response surface plot (right) and its contour plot (left) of interaction between Luegmi and Na2HPO4 concentrations with fermentation time kept at 24 hours.




## Figure 2

Pattern of BI2 lipopeptide biosurfactant production visualized by surface tension decreasing power: Response surface plot (right) and its Contour plot (left) of interaction between Luegmi concentration and time with a constant Na2HPO4 kept at 0.1 %.

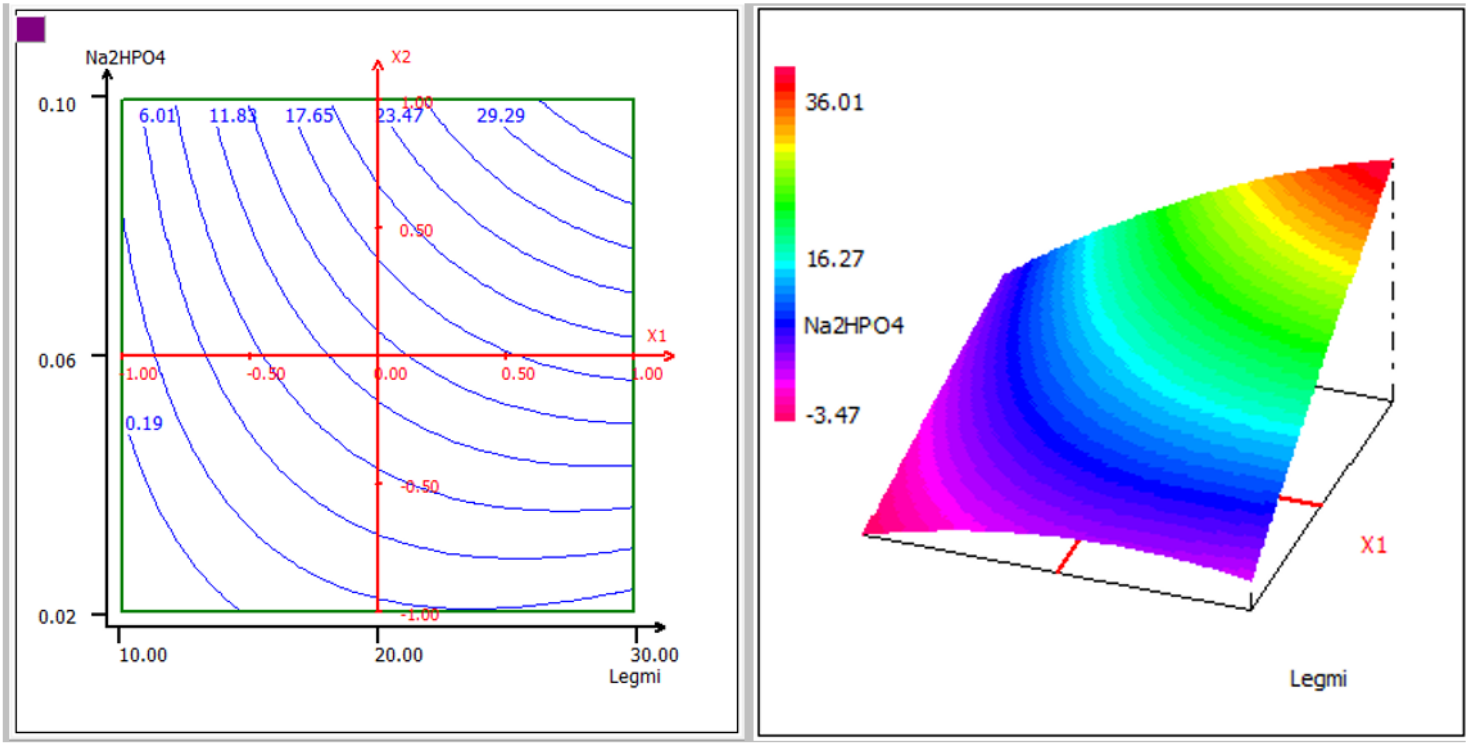



**Figure 3**

Pattern of BI2 lipopeptide biosurfactant production visualized by dispersing surface: Response surface plot (right) and its Contour plot (left) of interaction between Luegmi and Na2HPO4 concentrations with a constant time kept at 24 hours.

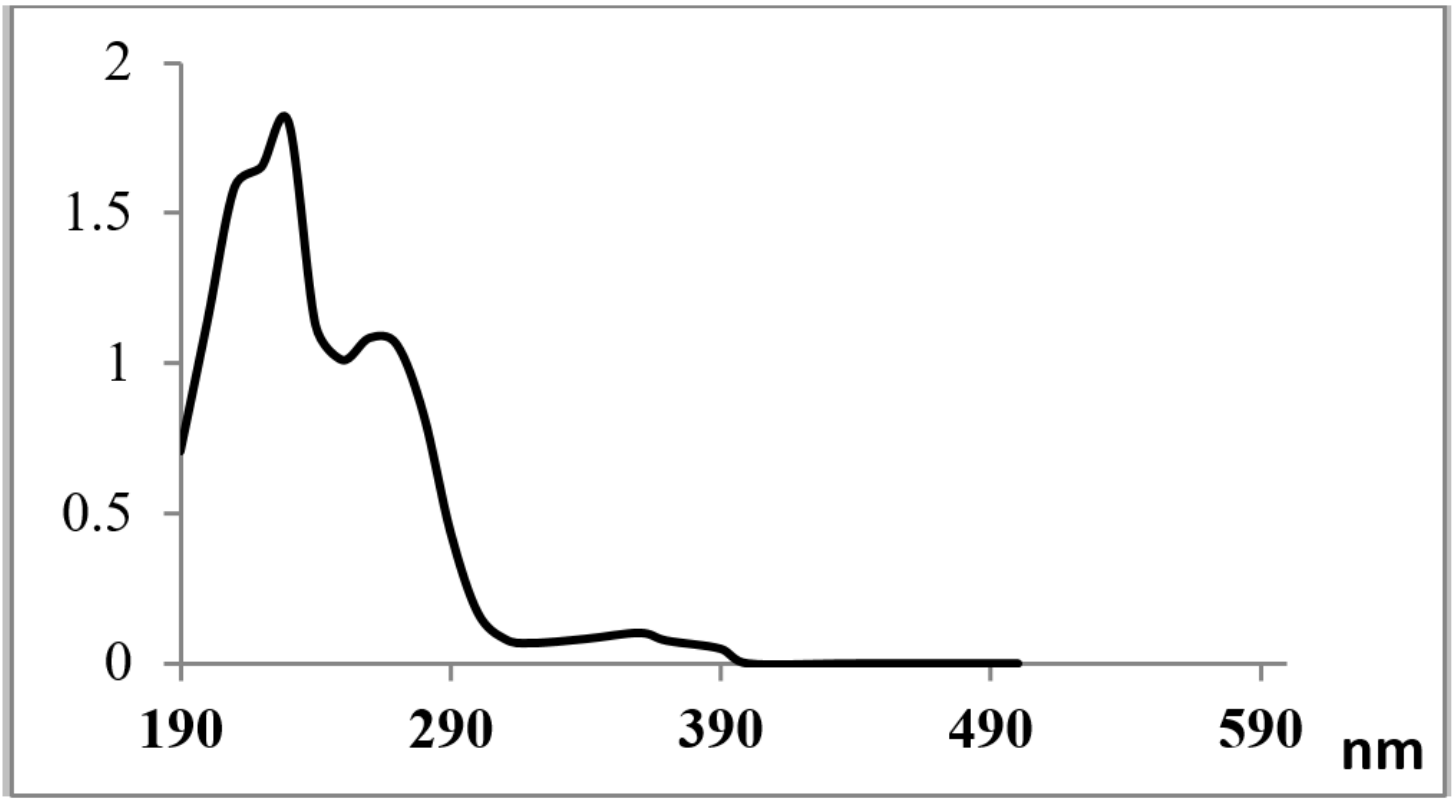
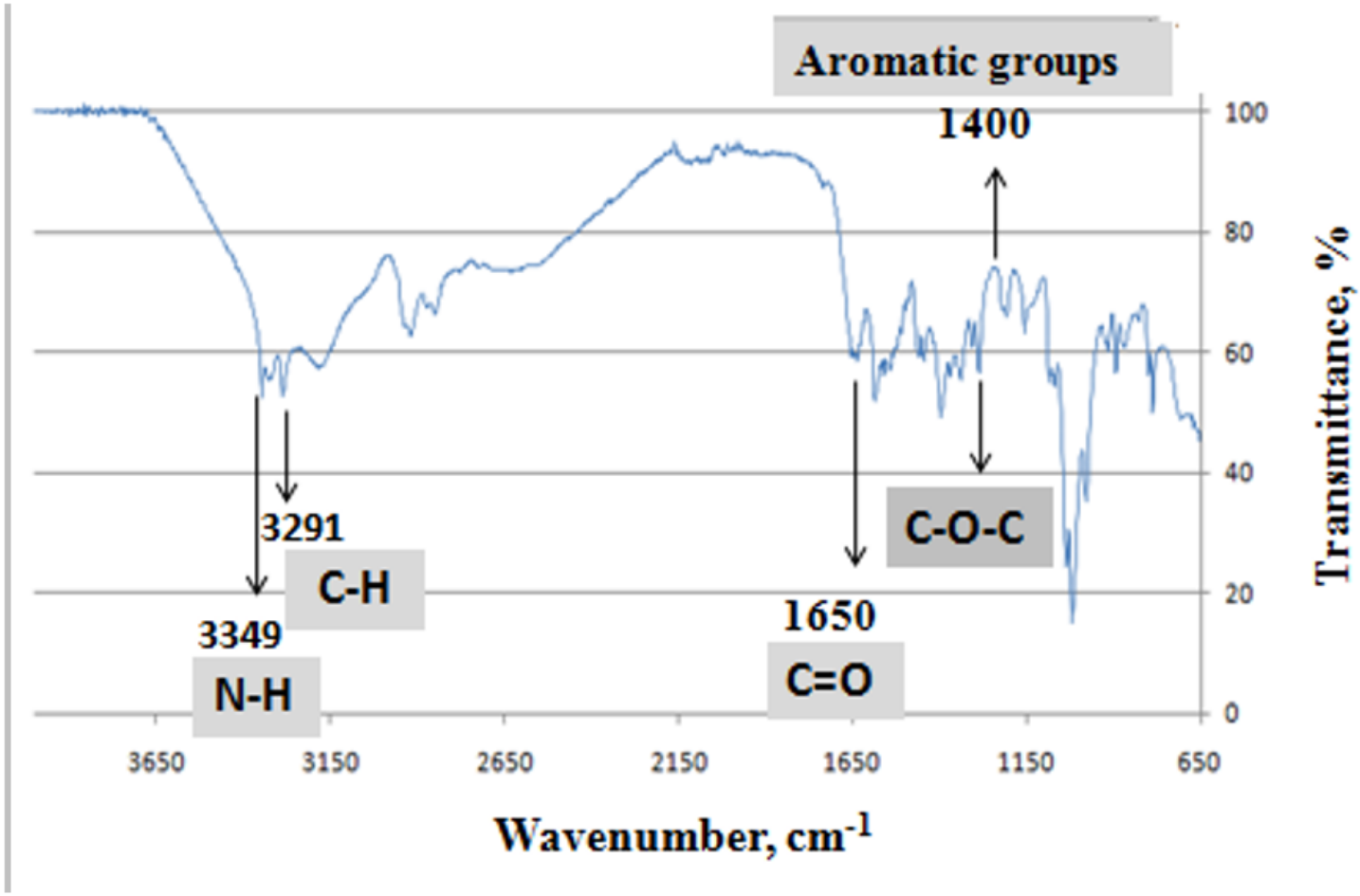



**Figure 4**

UV absorption spectrum of crude lipopeptide preparation of BI2 biosurfactant




## Figure 5

Infra-Red absorption spectrum of crude lipopeptide preparation of BI2 biosurfactant

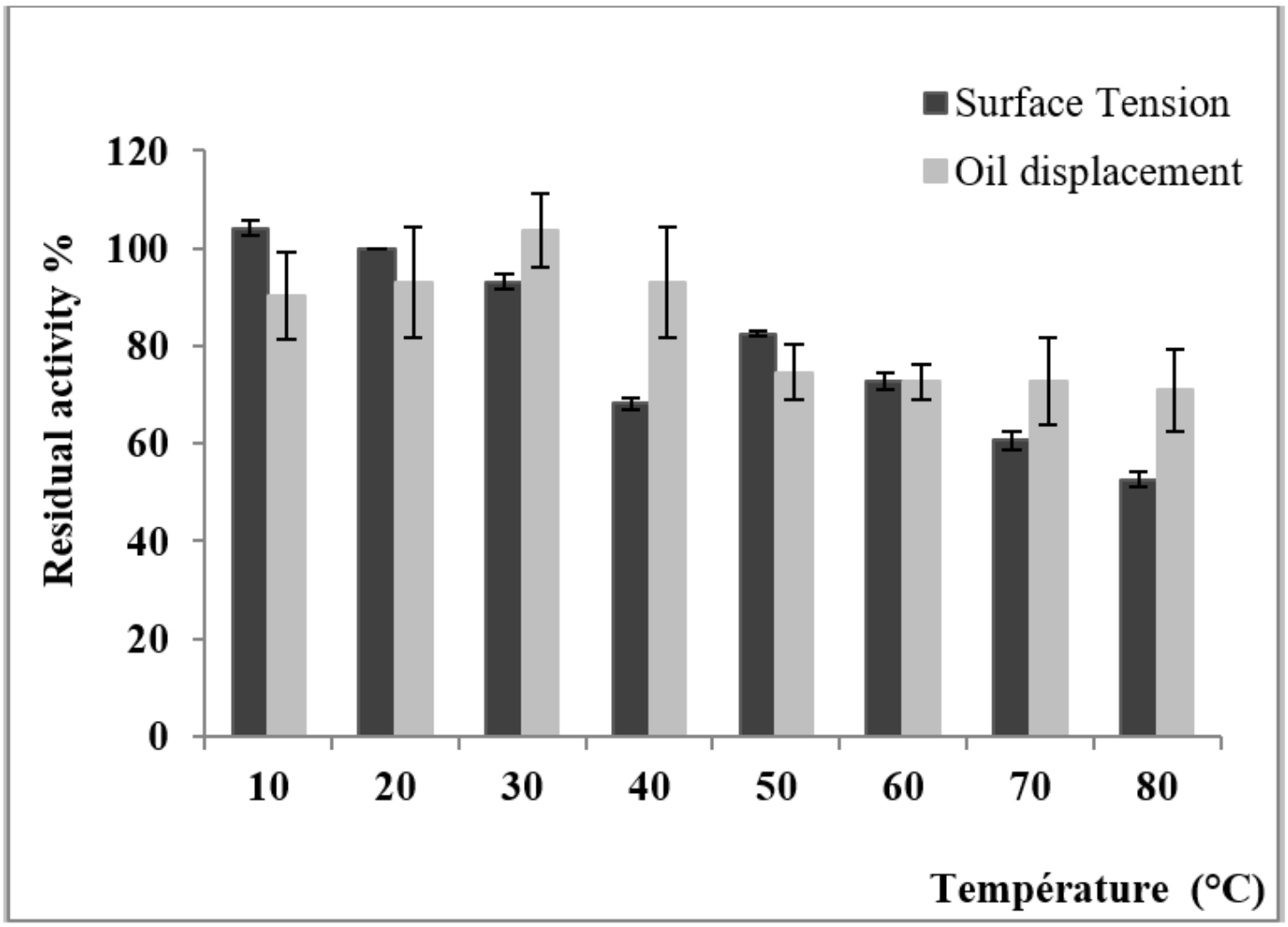



**Figure 6**

Effect of temperature on BI2 lipopeptide biosurfactant activity

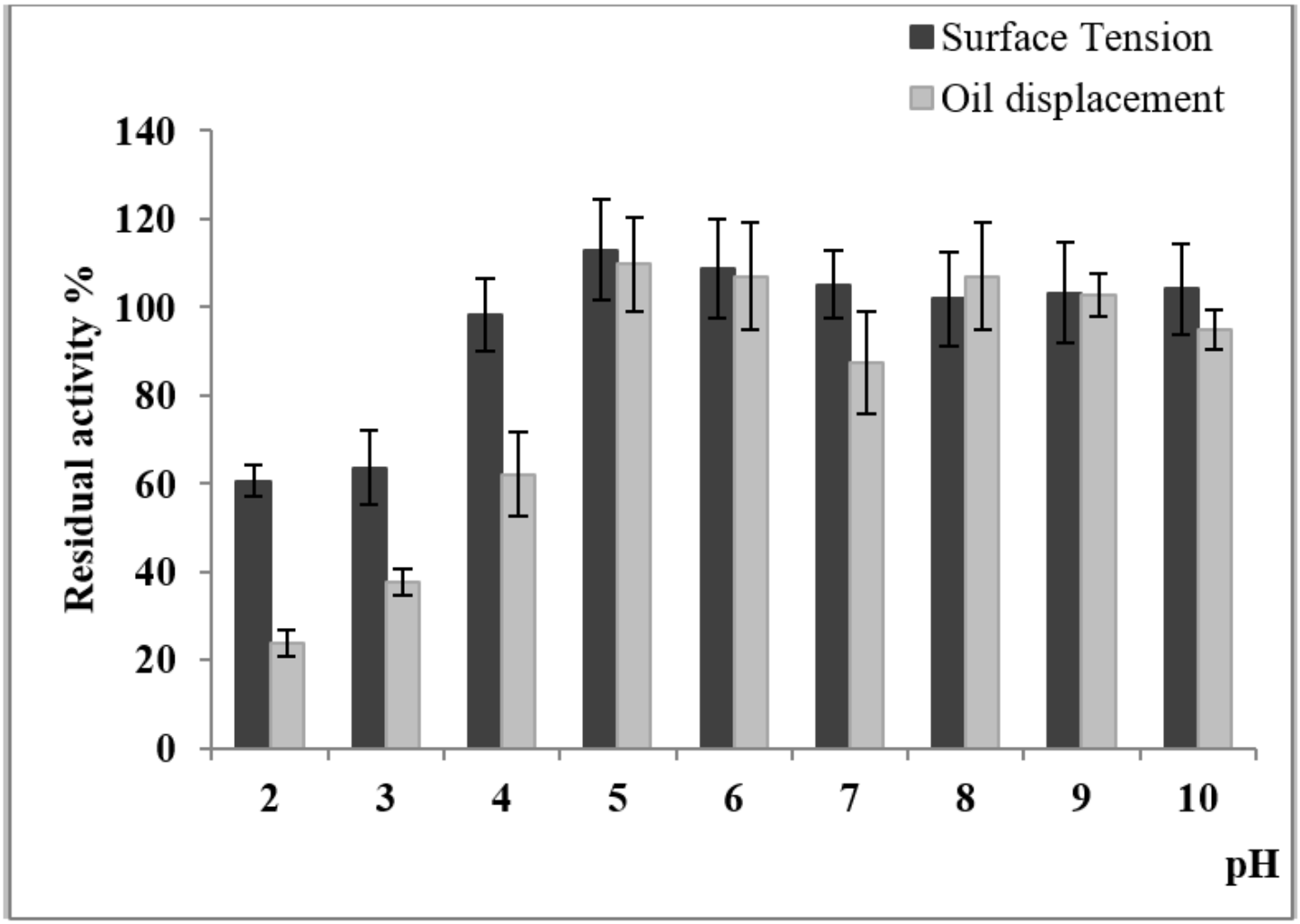



**Figure 7**

Effect of pH on BI2 lipopeptide biosurfactant activity

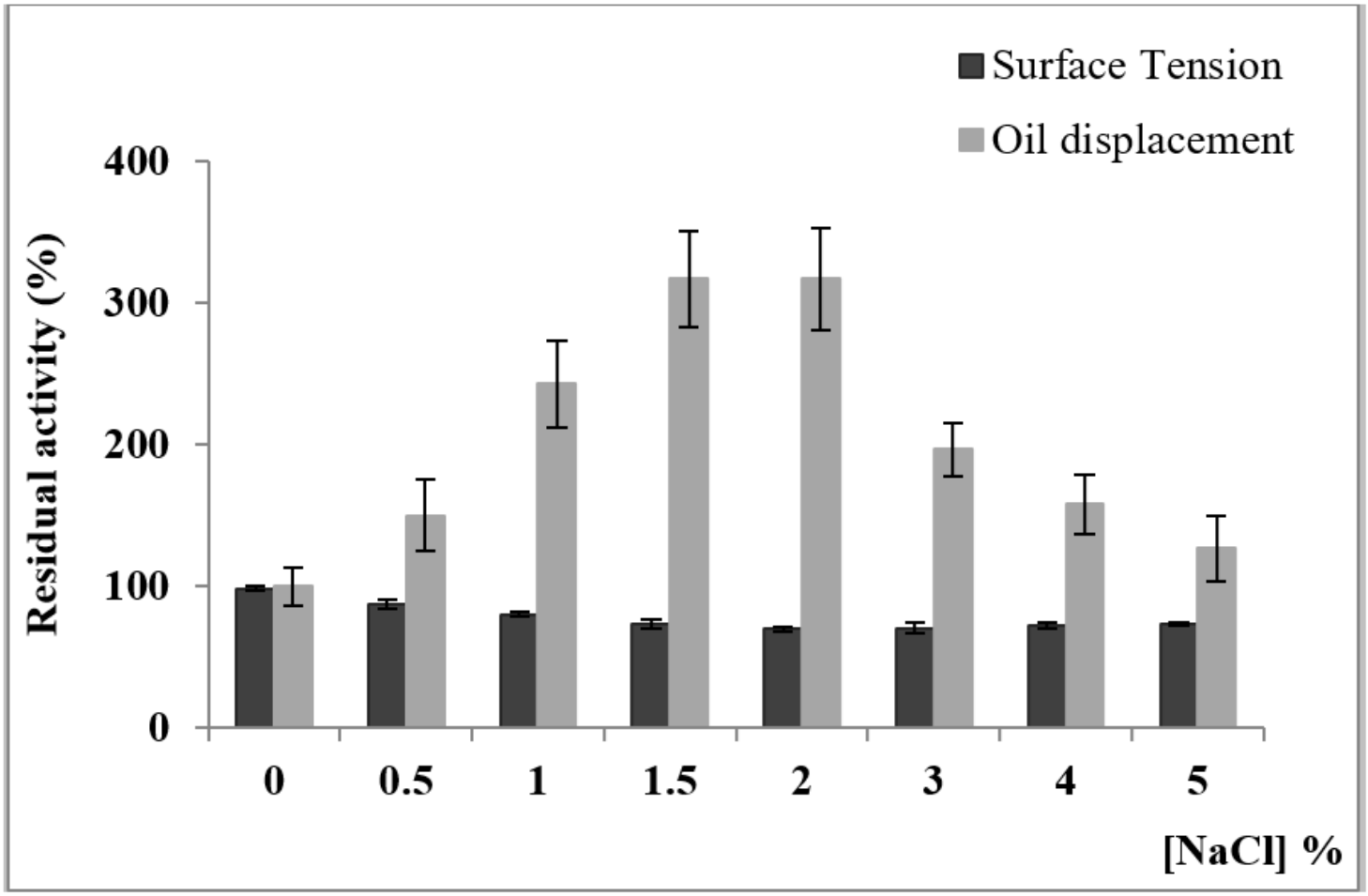



**Figure 8**

Effect of NaCl on BI2 lipopeptide biosurfactant activity

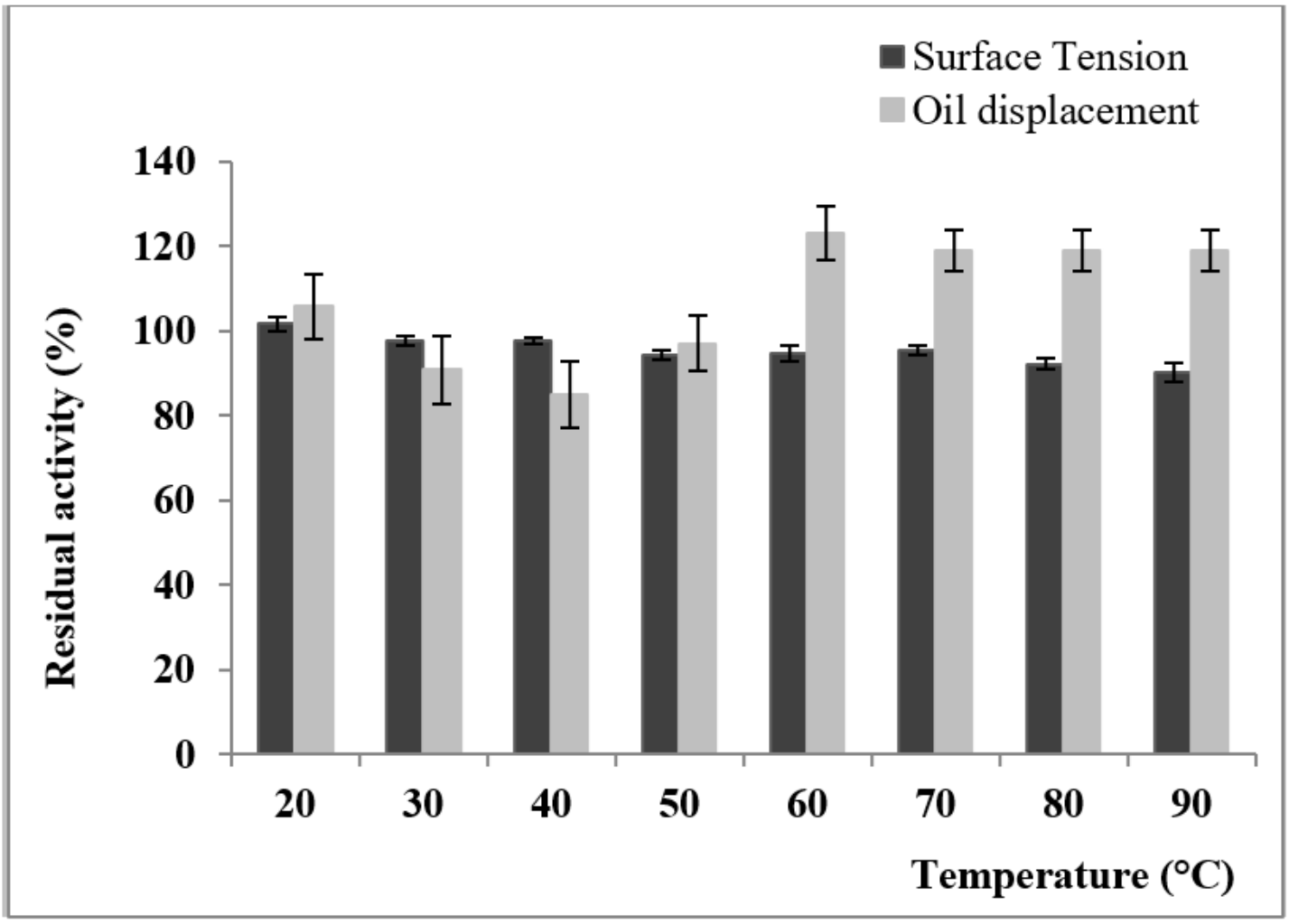



**Figure 9**

Effect of temperature on BI2 lipopeptide biosurfactant stability

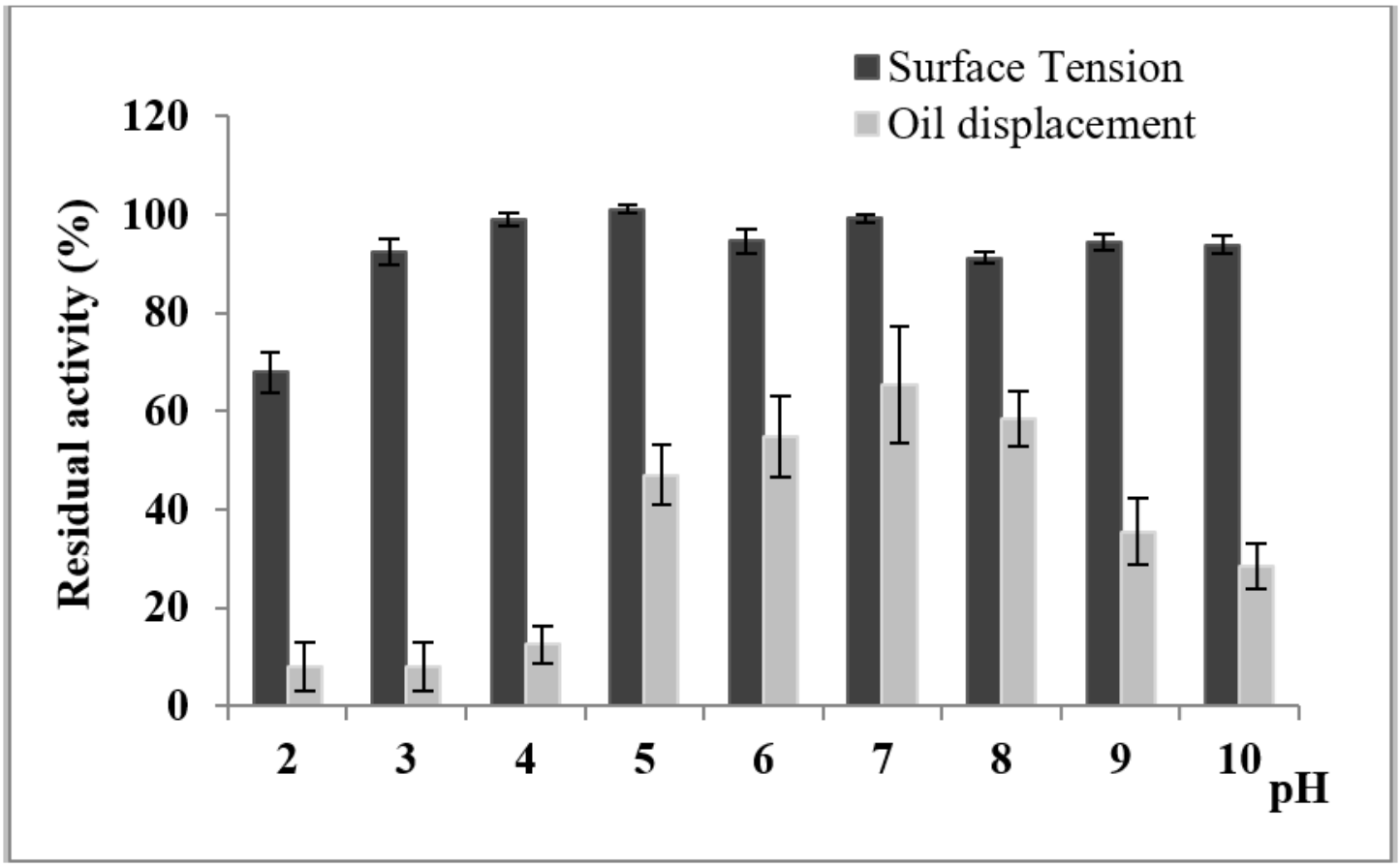



**Figure 10**

Effect of pH on BI2 lipopeptide biosurfactant stability

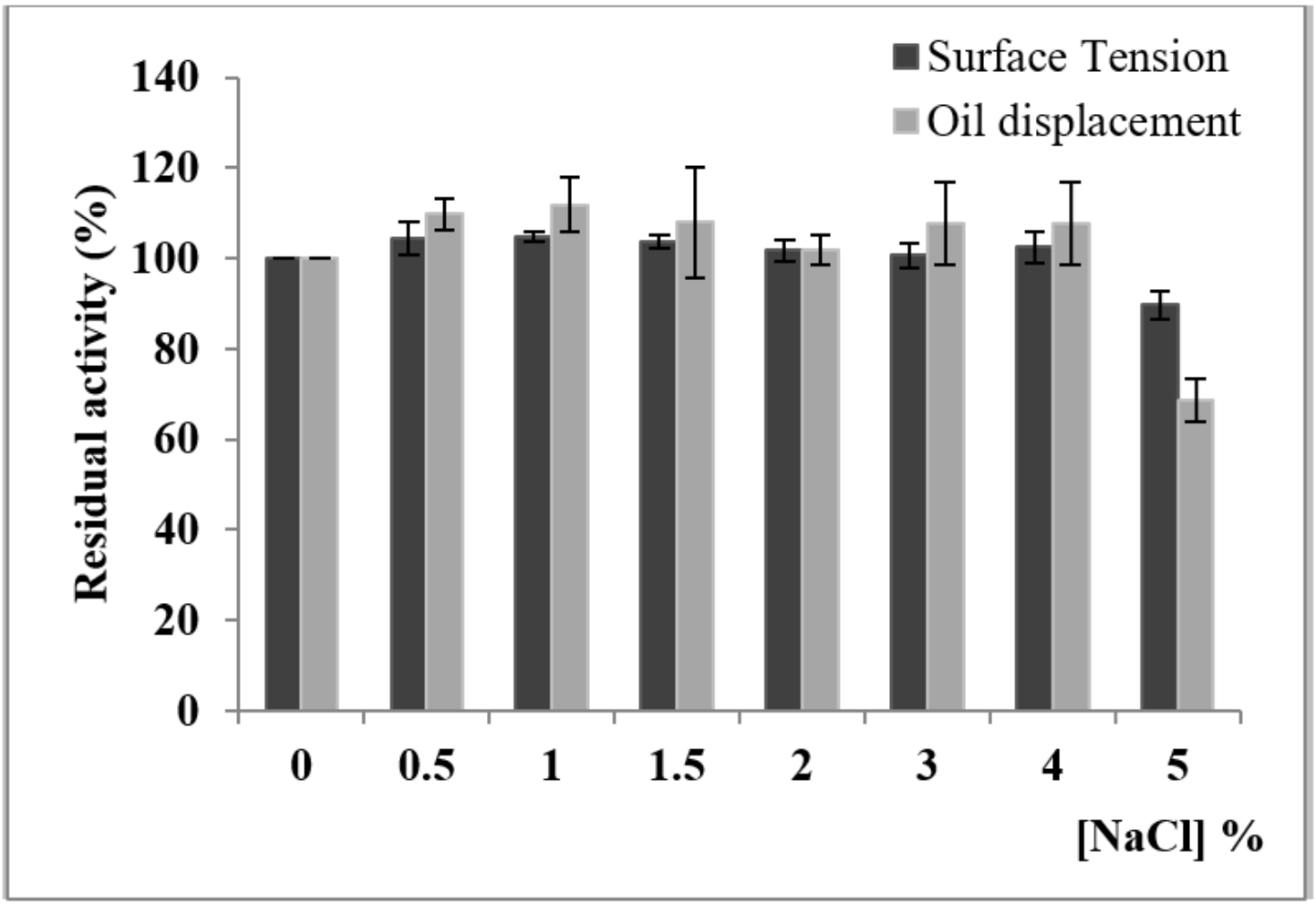



**Figure 11**

Effect of NaCl on BI2 lipopeptide biosurfactant stability

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstractBI2.tif